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EDITORS

CHARLES A. SHULL

CHARLES B. LIPMAN
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FRANCIS E. LLOYD

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ERRATA

First cover page, vol. 4, no. 1, in first title, for on, read in.
Page 136, first footnote to table, for *Gingko*, read *Ginkgo*.
Page 152, legend to figure, for $FeSO_4$, read K_2SO_4.
Page 157, line 13, for 1868, read 1865.
Page 160, citation 4, for sans, read sous.
Page 277, line 11 from bottom, for produces, read produce.
Page 278, line 13, for considerable, read considerably.
Page 279, citations 16 and 17, for chemiques, read chimiques.
Page 287, last line, for build, read built.
Page 302, footnote 1, for SAYBOLT, read SAYBOLDT.
Page 303, title of table II, for transportation, read transpiration.
Page 315, last column of table IV, sixth item, dele one word end.
Page 316, line 10 from bottom, for droppd, read dropped.
Page 403, line 12 from bottom, for $9.00, read $6.50.

Vol. 4 No. 1

PLANT PHYSIOLOGY

JANUARY, 1929

STUDIES IN EXPERIMENTAL CYTOLOGY

CHARLES F. HOTTES

(WITH FOUR FIGURES AND ONE PLATE)

I. Introduction

Cytologists have been too long content with the descriptive phase of their science. Cells of widely different character, and, consequently, in most diverse states or phases of physiologic activity, and prepared for study by the application of sundry methods, have served them for morphologic study and physiologic interpretation. This is responsible, in no small measure, (1) for the widely different results occasionally reported by different investigators working with supposedly identical material, and, (2) for the conflicting views now held with reference to the nature and the mechanism of the spindle, the behavior and function of the nucleoli, etc.—cell structures that have been the object of study since the beginning of the science.

The development of experimental cytology in recent years bids fair to lead us along lines of research affecting the intimate relation between the function and the structure of the cell organs, and through this relation to the discovery of the principles underlying cell activity. It has become quite generally recognized that the most noteworthy achievement in the progress of cell-study concerns, as one of the pioneers in experimental cytology says, "die Methode der Forschung. Lange Zeit über eine rein deskriptive Wissenschaft, versucht die Zellenlehre in der Neuzeit den exakten Wissenschaften, Physik und Chemie, nachzueifern, die Erscheinungen nicht nur zu beschreiben, sondern ursächlich zu begreifen, einerseits auf dem Wege des Experiments, anderseits durch die Anwendung exakter Messmethoden" (**13**, p. 2).

In following this newer line of cell-study one should never lose sight of the fact that during its period of activity the cell is subjected to a continuous series of chemical and physical processes induced by internal, and modi-

fied by external conditions or states. In the differentiated cell these follow a definitely fixed cycle in accordance with its specific functional activity; in the undifferentiated cell they pass through a regular progression of intercorrelated steps or phases as it advances to specific functional differentiation. Concomitant with the more or less fixed physiologic state of the differentiated cell, and the successive states of a meristematic cell in its progress toward physiologic fixity, may be noted a considerable morphologic detail. In the meristematic cell progressing toward specific functional differentiation the structure is constantly changing, and becomes fixed only when the cell assumes the permanent function demanded of it as a unit of the organism. This unit, in a given physiologic phase and under conditions such as usually prevail, possesses a specific structure and function which we call normal. The normal, in so far as it interests the cytologist, is the expression in cell structure of the harmonic interaction of the several cell organs. This interaction in the differentiated cell differs from that in the differentiating cell, and the cytologist is confronted by intergrading structures that are difficult or impossible correctly to interpret from a study of cells in a single physiologic phase.

The structure and function of an egg-cell is not that of a ganglion-cell, and the cell from the formative region of the root differs from one in the region of elongation. The degree and manner of reaction of these cells to chemical and physical agents, in so far as it can be followed in discernible structure and manifest function, is determined by the physiologic state or phase of the cell at the time of stimulation or subjection. But those conditions, chemical and physical, internal and external, which enable the cell, in consequence of the harmonic interaction of its organs, to perform its normal functions, that is, maintain the optimum of *cell* activity, are by no means identical with the conditions at which the *several cell-organs* in turn find their optima. The comparatively wide difference in the position of the cardinal points of activity—minimum, optimum, maximum—of the several cell-organs may be readily demonstrated by appropriate experiments. In the data later submitted and in the discussion that follows, it is apparent that the cytologist may, by carefully directed and controlled experiments, so affect the different cell-organs as (1) to excite or accelerate the activity of one while depressing or retarding that of another; (2) to modify completely the normal structure and function of one or several; and (3) to suppress the activity and destroy the structure of one or several. This furnishes an admirable opportunity to study the interrelations and interdependence of the several cell-organs, and must prove helpful in our endeavor to solve the intricacies of cell-processes, and of understanding cellular derangements (pathologic) resulting from lack of proper coordination.

Plant physiologists have long recognized that the manner of response of meristematic cells to stimuli is influenced or determined by the physiologic state or phase of the cell at the time of stimulation. SACHS (**38**, p. 220) was the first clearly to recognize this important fact, and expressed it as follows: "Das Organ ist heute nicht mehr dasselbe Ding wie gestern und wird morgen wieder ein Ding mit anderen Eigenschaften sein, selbst wenn man dies an der äusseren Form und der mikroskopischen Struktur nicht wahrnimmt. Wird nun das sich entwickelende, wachsende Organ von äusseren Einwirkungen betroffen, welche die Art seines Wachstums verändern, so muss diese Reaction verschieden ausfallen, je nach dem die gleiche äussere Einwirkung das wachsende Organ gestern, heute, oder morgen trifft."

Recent studies in experimental cytology and those here reported, most clearly demonstrate that the above statement applies equally well to the cell organs, and that in the future, greater consideration will be given to the physiologic state of the cell—tissue or egg—at the time of its fixation, or of its subjection to the action of external agents. Most of the work in experimental cytology has been done on cells in vastly different physiologic states, and the effect upon them of now one and now another stimulus of limited range of intensity and duration of action noted. This procedure gives us a mass of isolated detail, interesting and valuable in itself, but difficult or impossible to correlate. What we most need are studies that will enable us to coordinate the mass of unharmonized material we now have. Progress along this line of cytologic study is necessarily difficult and slow. For the present, we may profitably apply ourselves chiefly to an accurate and systematic experimental study of the behavior, toward physical and chemical agents, of the several cell-organs in cells of different physiologic states.

II. Materials and methods

The root-tip of *Vicia faba* was chosen because of its easy culture, its vigor, the readiness with which it responds to stimuli, and the large size of its cells and nuclei. Furthermore, it has been extensively used in cytologic study, and the recent investigations by LUNDEGÅRDH (**24, 25, 27**) on living material, give opportunity to appraise the respective values of results obtained by widely different methods of preparation for study. In a number of instances, the root-tips of *Zea mays*, *Phaseolus multiflorus*, and *Cucurbita pepo* were used for comparison. In the discussion that follows, all references, unless otherwise stated, pertain to the cells of the root-tips of *Vicia faba*.

GERMINATION

The seeds of *Vicia faba*, with the seed-coats previously broken to insure a more uniform swelling, were placed in tap water at 18 to 20° C. for 24

hours, the water being changed once. The swollen seeds were then placed in close proximity in a vertical position, with the micropyle lowermost, in a flat containing loosely packed, well-leached, moist sawdust. They were lightly covered with the same material, over which there was then spread a thin layer of dry sawdust to prevent excessive evaporation. A bed so prepared required no further watering and insured a rapid and normal germination. In from 50 to 60 hours at a temperature of 18 to 21° C., when the roots were from 3 to 4 cm. long, the seedlings were transferred on small-meshed paraffined wire netting to a jar of tap water for the purpose of accommodating the roots to an aqueous medium. The cotyledons resting on the wire netting immediately above the water of the jar, retained all necessary moisture for active translocation of materials by inverting over them a shallow second vessel of the same diameter as the jar. Diseased gelatinous tips, such as SACHS encountered when roots of *Vicia faba* were cultivated in well water for several hours, were never met with. For treatment only those roots that showed approximately the same rate of growth were used. The experiments were usually begun at eleven o'clock in the morning, since preliminary examination showed that the mitotic figures were then most abundant. At the beginning of each series of experiments, three of the root-tips of the seedlings about to be used were fixed as checks for comparison.

In an experimental study as here attempted the method of procedure is of primary importance, and the one outlined below was finally adopted and strictly followed. It is the direct outgrowth of unsuccessful attempts at correlation of results obtained by treating for a limited time, cells in different physiologic states, now with one, now with another agent.

THE PHYSIOLOGIC STATE

Cells of the same kind and origin but in different physiologic phases, were simultaneously subjected to the action of some physical or chemical condition or agent. The root-tip of *Vicia faba* with its clearly defined regions of cell multiplication, cell elongation, and cell differentiation, furnishes these conditions admirably.

THE INTENSITY OF THE ACTION

The physical and chemical conditions or agents were applied or supplied through a sufficiently wide range of intensity to obtain clear evidence of their specific effect on the cell organs, and to determine, when possible, the state of excitation or depression produced. By a sufficient extension of the intensity of the treatment, it was possible to determine the wide difference in reaction, adaptation, and finally, death-point of the several cell-organs;

and to observe differences, if any, in the manner or degree of response in the same organ in its different physiologic phases.

The time or duration of action

It is a well established fact in general physiology that the time, that is, the duration of action of an external agent is a factor of considerable importance. To know the effect of the agent applied for a brief period is not sufficient. We must learn something of the manner in which a cell-organ adjusts or adapts itself under certain prolonged conditions of action, and how these adjustments, in turn, affect, through correlation and interdependence of the organs, cell function and structure. Hence, in the experiments, the time or duration of action of the stimulus or agent was sufficiently prolonged (1) to allow a complete adaptation; or (2) to lead to complete exhaustion; or (3) to cause the destruction of a cell-organ.

To follow this cumulative effect it is necessary to use a large number of seedlings (100 to 200 for each series) and, while the conditions of the experiment are accurately maintained, to remove and fix at predetermined intervals, the root-tips of a given number.

Restitution

Whenever from an experimental series roots were fixed for study, from 9 to 30 seedlings, similarly treated, were transferred to tap water at 18 to 20° C. for restitution. The successive stages in the process of restitution were followed by fixing three root-tips of the transferred seedlings at intervals of from 10 minutes to 1 hour. This phase of the experimental work has proven most interesting and has been a great help in the elucidation of some of the complex changes induced by the previous treatment.

Necrobiosis or necrosis

When seedlings from a member of an experimental series transferred to water for restitution, showed, through death, that the damage done by the treatment was irreparable, *all* roots of that member in the series were excluded from further consideration. No attempt was made to follow the necrobiotic or necrotic (**45,** p. 162, 407, 412) changes in the severely injured cell, and such phenomena are not here considered. Our literature is by no means free from descriptions of such "submortem" (**11,** p. 160) structures.

Sections of living root-tips

Early in the development of our science Flemming, Bütschli, and others, recognized that the appearance of the several cell-structures, but more particularly that of the cytoplasm, may be due to the action of the

fixative or to the gradual changes occurring in the cell as it passes through successive stages from life to death—necrobiosis.

Later, with the idea of protoplasm as a colloid rapidly developing, the interesting and valuable experiments of HARDY (**11**) and FISCHER (**6**) firmly established this view, and, by the production, artificially, of structures appearing like those generally described by cytologists, placed in question much of the detail of cell-morphology and physiology.

Following HARDY and FISCHER we find a growing tendency to minimize the cumulative results obtained by cytologists using the fixation technique. By resorting to ingenious and skilful manipulation of materials bearing no direct relation to protoplasm, LEDUC (**23**, p. 121) and others have produced and explained (?) the mitotic process through the phenomena of osmosis, diffusion, coagulation, etc. LUNDEGÅRDH, paying little or no attention to these physio-chemical aspects, believes that the real solution of cell-structure must rest with the study of the living cell. By following this method only can the cytologist overcome and correct the errors for which his faulty technique is largely responsible.

In entering upon an extensive study of the influence of physiologic phase, and of physical and chemical agents on cell-structure, it becomes imperative that a technique above reproach should be employed.

From the criticisms made regarding the shortcomings of the fixation method, and the general recognition of the justification of such criticisms by cytologists, there seemed but one alternative, namely, the use of the direct method of observation in living material, or, at any rate, the use of living material as a check or arbiter in the interpretation of structural detail. Accordingly a serious and extensive study of living sections from both normal and treated root-tips was undertaken. The results from such a study were extremely disappointing, but it was thought that perseverance and training in the use of the new method would finally overcome the difficulties and lead to success. This optimism proved ill-founded, and after repeated trials and comparisons, the study from living material was abandoned. This, to some, will seem like heresy, and disregards the dictum of LUNDEGÅRDH (**24**, p. 237) "Und doch sind wohl keine wahren Fortschritte möglich, ohne dass man auf dem zuverlässigen Grund des direkt beobachteten baut."

The reasons that prompted the abandonment of the method of direct observation will be fully discussed under a separate heading. A few quotations from LUNDEGÅRDH's contributions will give, through admissions, some of the more apparent reasons, and will, it is hoped, hold prejudice in abeyance. "Über die allerfrühesten Stadien lässt sich am lebenden material keine bestimmte Auffassung bilden." (**24**, p. 275.) "Wenn auch—wie

gesagt—am lebenden Material sich eine ziemlich lückenlose Auffassung der Morphogenese des Karyotins während der Kernteilung gewinnen lässt, so ist doch die erzielte Erkenntnis nicht vollständig, und tatsächlich lässt einen das lebende material im Stich bei einer Anzahl wichtiger Vorgänge und Erscheinungen, wie z. B. der Metakinesis und den Anfangsstadien der Längsspaltung in den Spiremfäden." (**24**, p. 237.) "Die Art des Auseinandergehens der Chromosomen, der Teilung der Kernplatte, hat man nicht im Leben feststellen können." (**24**, p. 277.) "Sogar ganze Kerne können infolge der Lichtbrechungsverhältnisse unsichtbar sein und durch Hinzufügen von Mitteln, die die Wasserimbibitionsverhältnisse oder den Chemisch-physikalischen Zustand der Zelle verändern, sogleich zum Vorschein gebracht werden. In den Ruhekernen von *Vicia faba*, die in der Streckungszone liegen, sieht man häufig nichts ausser den Nukleolen. Nach Fixierung erweisen sie sich mit Karyosomen und Gerüst versehen, wobei Zahlenverhältnisse und Analogien mit den übrigen Kernen entschieden für die Präformation der ersteren sprechen." (**27**, p. 252).

Fixing and staining

In order that the effects on the cell of the various agents employed throughout this series of experiments should be strictly comparable, a single fixing agent, Hof's (**14**, p. 67) modification of Flemming's chromic acid mixture, and but one stain, Flemming's safranin, gentian-violet, orange combination, were used. Of the Flemming mixture Lundegårdh (**25**, p. 214) says, "Ich habe in einigen Fällen den Vorgang unter dem Mikroskop verfolgen können, und grössere Umordnungen schienen wirklich dabei nicht einzutreten (ich spreche fortwährend von der Flemmingschen Flüssigkeit)."

To aid in interpretation, and to check the method used in fixing and staining, the following were, in special cases, used: O. von Roth's, Gilson's, and Zenker's fixing agents, and Zimmermann's fuchsin and iodine-green, Heidenhain's iron haematoxylin, and methylgreen. Frequently sections of treated and normal roots, of roots fixed in Hof's mixture and some one of the fixatives above mentioned, were placed together on the same slide and stained by one or another method. This was done to determine the action of the fixative and stain on normal and treated cells, and, more particularly, in the study of nucleoli, etc., to guard against "Spiegelfärbung" (**6**, p. 197).

The material remained in the Hof fixing agent for 30 hours, and was then washed in running water for 5 hours. It was dehydrated in alcohol of increasing concentration, cleared in chloroform, and imbedded in paraffin of 52° C. melting point. The sections were cut in ribbons, as a rule 5 μ in thickness, and fixed to the slide with Mayer's albumen fixative.

III. The cells of the normal root-tip

The cells of the normal root-tip when subjected to the various external agents and conditions later described, frequently show marked changes in structure and behavior. That these changes may be more readily recognized, a brief account of the cells of the normal root-tip is here given.

ZIMMERMANN (**48**), LAVDOWSKY (**22**), ROSEN (**36**), HOF (**14**), HOTTES (**16**), and GARDNER (**8**), described the metabolic, interphasic, and mitotic cell phenomena of *Vicia faba*. Although these earlier studies have been extended and amended by the work of FRASER and SNELL (**7**), of LUNDEGÅRDH (**11, 19, 24, 25, 26, 27, 28**), of SHARP (**41**), of SAKAMURA (**40**) and others, there still remain a number of points in controversy both as to detail of structure and interpretation. I feel confident that the experimental method used in the parts to follow will contribute evidence useful in reaching tenable conclusions regarding some of these mootable cases.

The manner and degree of structural change and functional reaction which the normal cell when subjected to chemical and physical agents undergoes, depends upon the physiologic phase of the cell at the time of stimulation. Hence a characterization of the cells of the three regions of SACHS is of primary importance. The region at the very tip with nearly trimetric cells filled with non-vacuolate cytoplasm, and with nuclei commonly in active mitotic division, is the region of cell formation; following it, with cells distinctly elongated, cytoplasm characteristically vacuolate, and with nuclei less commonly in mitotic division, is the region of elongation; finally with the elongated cells of the region just described, undergoing through internal differentiation the specific changes that lead to their mature structure and permanent function, is the region of differentiation. Since the above division of the cells of the root-tip into three regions is based entirely on the physiologic state of the cells, as was the intention of SACHS, it is impossible to locate the limits of the respective regions by distance from the tip of the root or the number of internodes from the tip of the stem. Failure to recognize this has led cytologists and ecologists into grave error. The length of the first and second zones is established by the influence of the factors of the environment on growth in length. Under the most favorable growth conditions the region of differentiation may be several centimeters removed from the tip, while under unfavorable conditions it is pushed acropetal to within a millimeter.

The cells of the formative region are filled with a dense, flocculent cytoplasm, near the center of which lies a spherical nucleus somewhat smaller than that of the slightly older cells. This interphasic nucleus has a reticulum of small meshes, somewhat irregular in form and size. Over this reticulum smaller and larger particles of chromatin are distributed. The

larger particles of irregular form, undoubtedly are identified with the bodies that LUNDEGÅRDH describes as karyosomes. They are by no means constantly present, and as SHARP (**41**, p. 309) has observed, show no longitudinal splitting as LUNDEGÅRDH reported. Occasionally the slightly irregular reticulum above referred to, is due to the faintly distinguishable outlines of the telophasic chromosomes in the process of transformation, as SHARP maintains. The varied appearance of the interphasic nucleus in the formative zone as well as in others, LUNDEGÅRDH (**25**, p. 228), is merely the morphologic expression of the rapid changes it undergoes from one physiologic phase to the next. In the experimental section will be noted the intimate and direct relation between these phases and the reaction of the cell to chemical and physical stimulation. Contrary to the statement of ROSEN (**36**, p. 265), the nucleus has a very distinct nuclear membrane. One or two nucleoli of spherical form, and never larger than one-third the diameter of the nucleus, lie in the center of a clearly defined hof. This hof differs in size, occasionally attaining a diameter equal to one-half that of the nucleus. Although very generally present in prepared material, it has been called an artifact by most cytologists, due to the refractive powers of the spherical nucleolus or to its shrinkage through poor fixation. Notwithstanding, the hof is here described as an intrinsic part of the structure of the nucleus and the convincing evidence of its reality will be presented in the experimental section. Mitotic figures in all phases are abundant and will be described later.

In the region of elongation the cells have sufficiently differentiated to enable one clearly to distinguish between the three rudimentary tissue systems. The cells of the dermatogen soon lose their meristematic character and simply elongate. In the younger regions these cells are still filled with a rather dense cytoplasm which becomes vacuolate as you pass to the next older. The nucleoli, one or two in number, are always small and frequently have a relatively large vacuole. The hof is either very small or is absent. The cytoplasm of the enlarging cells of the periblem increases very slightly or not at all and becomes vacuolate. A short distance beyond the typical formative zone, the greater part of the cell is occupied by a single vacuole. The nuclei in the younger portions of this zone reach their maximum size and remain nearly spherical until flattened by the pressure of the enlarging vacuole. The nucleoli also are larger than in the formative zone, though the increase is not in the same ratio as that of the nucleus. A hof is present in the cells of the periblem bordering on the formative zone. In the slightly older cells it is not uncommon to find threads of the reticulum to cross the hof to the nucleolus. Later chromatin granules may be found deposited on these threads. NĚMEC (**32**, p. 316) and others have

described similar threads crossing the hof. A lobed or toothed nucleolus resulting from the intimate contact of the threads with the nucleolus as LUNDEGÅRDH (**24,** p. 256) describes, was not observed; nor was a penetration of the nucleolus by the threads as stated by GARDNER (**8,** p. 174).

The cytoplasm of the cells of the procambial strands in the transition zone of the region of elongation, is flocculent and markedly denser than that of the younger cells of the root-tip. The nuclei in the long and narrow cells are usually elliptical in form, of large size, and furnished with a very dense reticulum over which numerous chromatin bodies, chromocentra, of varying size and shape, are distributed. The nucleoli are large, frequently elongated or lobed, and have one or more large vacuoles. A well defined hof is usually present. Amitoses as described by LAVDOWSKY (**22**) are absent.

The metabolic nucleus, virtually absent from the zone of cell formation, becomes increasingly prevalent in the older regions of the zone of cell elongation and of certain tissues of the zone of cell differentiation. It is in these regions, if anywhere, that its typical form should be sought, and not in the cells of the root-cap, LUNDEGÅRDH (**25,** p. 228). The cells of the root-cap very early pass through a series of intermediate stages, necrobiosis, from normal functional activity to more restricted activity and death. So, too, when he states (p. 228) that typical metabolic nuclei may be found throughout the cells of root-tips imbedded for a longer period in plaster of Paris, he fails to recognize that they are typical only for the cells of roots so treated. In fact, as will be shown later, their structure differs vastly from the nuclei found in the normal root-tips.

The structure of the metabolic nucleus is correlated with the physiological functions that it performs and the nature of the tissue of which it is a part. In a measure, they differ from one another to the degree in which their functions differ. Even a cursory examination of a longitudinal section of a root-tip will disclose the difference in the metabolic nuclei in the different zones, or of epidermis and plerome. The nucleus of the epidermal cell with its rather delicate and uniform reticulum, few and small chromocentra, small nucleoli and hof, and often cyanophilic reaction, stands in marked contrast to that of the cell of the future tracheal vessel with its coarse and often irregular reticulum, numerous and large chromocentra, large nucleoli and hof, and erythrophilic reaction.

The chromocentra have been previously described as pseudonucleoli, accessory nucleoli, chromatin nucleoli, prochromosomes, chromocentra, karyosomes, etc. They have been made the object of a more extensive study both in the living and fixed cell, by LUNDEGÅRDH (**25**) who suggested the term karyosome. To the use of this term TISCHLER (**44,** p. 65) finds

objection, and uses the term chromocentra proposed by BACCARINI (**2**). The number and size of the chromocentra is undoubtedly influenced by the metabolic state of the cells of *Vicia*, as was observed by ROSENBERG (**37**) and HUIE (**17, 18**) in the cells of the tentacles of *Drosera*. The observations of ROSENBERG and others [TISCHLER (**44**) p. 65] that the number of chromocentra—prochromosomes—as in *Capsella* is the same as that of the chromosomes, is not true for *Vicia*. The reticulum of the metabolic nucleus in these regions of the root-tip shows no areas of denser nature such as SHARP (**41**, p. 310) described and assumed to represent the chromosomes of the previous telophase, but is like that of FRASER and SNELL (**7**) and of LUNDEGÅRDH (**25, 28**).

In the early prophase the reticulum of the interphasic or metabolic nucleus gives way to a single slender thread that is much convoluted and becomes more and more basophilic as its development proceeds. This transformation takes place in the interphasic nucleus of short rest period after the manner described by SHARP (**41**, p. 311). The anastomosing branches of the nuclear reticulum are gradually withdrawn while the area of the reticulum constituting the telophasic chromosome undergoes condensation. In the less favorable stages of the interphase, and, more particularly, in the metabolic nucleus where no areas that might be designated as the telophasic chromosomes are observable, the condensation takes place into what appears as a continuous thread and shows no distinct ends of the chromosomes. [TISCHLER (**44**), p. 113].

The thread formed by one or the other method is not of uniform consistency or composition. That it varies in consistency is universally recognized; that it varies in composition is one of the most debated of nuclear phenomena [WILSON (**47**), SHARP (**42**)], and most difficult of solution. Its solution, for obvious reasons, is impossible from studies *in vivo*, and stains and micro-chemical reactions seemingly have increased our difficulties. Experimental cell-physiology apparently is destined to furnish the key. In its earlier and slender stage the thread shows a distinct transverse banding as first observed by PFITZNER in 1881 and since described in numbers of cases in the spirem of both plant and animal nuclei. The existence of these basichromatic bodies, chromomeres, in regular alternation with oxychromatic fields in the thread, is challenged by a number of observers. TISCHLER (**44**, p. 312) once an ardent supporter of the chromomere theory, closes his discussion as follows: "VAN WISSELINGH (1899) sowie GRÉGOIRE (1906, 1907) und seine Schule, MARTINS MANO (1904), STOMPS (1910), LUNDEGÅRDH (1912c, 1913a), SHARP (1913, 1920b, 1921, S. 155) zeigten aber m. E. unwiderleglich, dass es sich dabei oft um eine Verallgemeinerung einzelner Zufalls-Strukturen handle." After such a strong statement it

would seem folly to reopen the question. But a critical examination of the evidence given by TISCHLER and others, by no means shakes the confidence in the observations made on the normal cells of *Vicia* supported and extended by the experimental results later to be discussed. Structures that have been observed *in vivo* (CHAMBERS); that reappear with distinctness and in constant alternate succession on the spirem before condensation in numbers of plants and animals; that may be separated by the pull of the micro-dissection needle (CHAMBERS); that occur and reoccur in characteristic sequence, form, and position through successive generations in the spirem of *Phrynotettix* [WENRICH (**46**)]; that support and are supported by theories of heredity, and confirmed by experimental studies in genetics, are by no means "Zufalls-Strukturen."

A splitting of the chromocentra and their transformation into paired threads from which the chromosomes are built up as LUNDEGÅRDH (**25,** p. 256) describes, was not observed. The chromocentra gradually fade away in the manner described by SHARP (**41,** p. 312) and the material is incorporated with that of the developing skein. The thread now undergoes a longitudinal splitting, though the manner in which this occurs is not clear. Median vacuoles appearing as narrow slits such as described by NOTHNAGEL (**33,** p. 453) were not observed. Vacuolization in the normal chromosome of the telophase as CHAMBERLAIN (**3,** p. 209) describes, is evident, and in some of the treated cells later to be described, extremely marked. Following the longitudinal fission the thread becomes shorter and thicker, and forms into a dense mass surrounding the nucleolus. In this stage all trace of a split is lost through temporary fusion. In preparations not too densely stained and with strong light, the nucleolus, somewhat reduced in size and frequently irregular in outline, may still be seen in the center of the mass. While in this intimate contact with the chromatin thread, it rapidly decreases in size and finally entirely disappears through solution. The rate of solution differs with the nature of the cell, and is greatly affected by the environment. The idea of ROSEN (**36,** p. 272) "vielleicht spielen auch hier Ernährungs- und Wachsthumsbedingungen der Organe, welchen die Kerne angehörten, eine Rolle" is confirmed in the experimental part.

While the thread shortens and thickens as described, the fundaments of the spindle appear in the form of delicate kinoplasmic structures usually on opposite sides of the nucleus. In these fundaments, at first low and dome-shaped, later conical, the kinoplasmic fibers may frequently be seen to diverge from several points (multipolar diarch), and later from a single point-bipolar diarch [STRASBURGER (**43**), p. 118]. According to DEVISÉ (**5**) and ROBYNS (**35**) these pole caps are of intranuclear origin and are intimately connected with the change in size which the prophasic nucleus

undergoes. With the shrinkage of the nucleus in the later spirem stage, a polar flattening takes place due to the exudation from the nucleus of part of its karyolymph. This karyolymph meeting with the cytoplasmic fluid forms, through gelation, a homogeneous mass, the polar caps. The presence of fibers in these caps is attributed by them and others, to bad fixation.

For the present I must adhere to the statement above made, and agree with ALLEN (**1**, p. 281) that the spindle is of both nuclear and cytoplasmic origin, and that the fibers are real and perform a definite function in the mitotic process. A fuller discussion is reserved until the experimental evidence has been presented. When the closely wound thread opens and flattens into the equatorial plate, the paired superimposed chromosomes lie in a single plane. Repeated counts showed 2 long and 10 short [SAKAMURA (**40**)].

The spindle is now completely formed and consists, contrary to DEVISÉ (**5**), of two sets of fibers, (1) those of the central spindle running from pole to pole, and (2) the mantle fibers running from the place of insertion on the chromosome to the poles. The insertion of the fibers is near the middle of the 2 long and near the end of the 10 short chromosomes.

Slight deviations from the normal position of the mitotic figure are not infrequent in the cells of the normal root. They are largely due to space relations. In the broad but relatively short cells of the periblem, the mitotic figures not infrequently are placed diagonally in the cell and the chromosomes of the equatorial crown may not lie in a plane. By the rotation of the phragmoplast in the process of wall formation, the cell wall is usually formed perpendicular to the long axis of the root. It is clear that in such cases the polar caps do not indicate the polarity of the cell as maintained by ROBYNS (**35**).

The anaphase and telophase have been very fully described by others. They shall receive further attention after certain features contributed by the experimental section have been described.

Part I

1. The effects of pressure on the cells of the root-tip

A. ROOT-TIPS IMBEDDED IN PLASTER OF PARIS BLOCKS

The seedlings were grown in a manner already described, and when the roots had reached a length of 2 cm., they were thrust into a rather stiff mixture of plaster of Paris and water, as described by PFEFFER (**34**, p. 239). The plaster of Paris block must not be too small or the pressure exerted by the growing root will cause it to burst. The seedlings, with roots so imbedded, were placed in shallow pans of water at 18° to 20° C., and fixed at intervals of 24 hours for a period of 30 days. It was found that it would

not do to free the root from the plaster of Paris block before fixing. When freed before killing the root elongates considerably, as will be described later, and thus disturbs the true relations of the cells and cell structures. To prevent this, the seedling, with the block somewhat pared down, was immersed in the fixing agent for several hours for initial fixing. The roots were then freed of the adhering plaster of Paris and placed in fresh fixative for the usual time.

The changes occurring in the cells of imbedded roots during the first two days are marked; after that the difference is one of degree only. For description the cells of a root-tip imbedded for five days has been chosen.

The cytoplasm in all of the cells is greatly reduced in quantity, and is of a delicate flocculent structure. Rather course fibers staining deeply with the gentian-violet, are frequently found irregularly distributed through it. Occasionally, structures of a similar nature form a number of concentric rings with a small dense body in the center (fig. 1, pl. I). The regions of meristematic cells have been reduced fully one-half, and, in many respects, the cells present an appearance not unlike that of the inanitiated roots, to be described later. The zone of elongation has correspondingly decreased, and the tracheal tissue has moved acropetal to within 2.5 mm. of the tip [PFEFFER (**34**), p. 351; NATHANSOHN (**29**), p. 680], and the fundament of the youngest lateral root, to within 5 or 6 mm. of the tip. In the large vacuoles of many of the prematurely aged cells lie heaps of bodies staining orange.

The nucleus is smaller than in the corresponding physiologic zone of the normal root. Its reticulum is very coarse, dense, and basiphilous; here and there the individual meshes seemed to be enlarged, but, closer examination shows that this apparent enlargement is due to a branch of the greatly modified hof. This branch can readily be followed through the reticulum to the nuclear membrane. Where it meets the nuclear membrane this is raised in the form of a blister (figs. 1 and 2, pl. I), and gives the nucleus an amoeboid appearance.

The greatly reduced nucleolus, 1/3 to 1/15 the diameter of the normal, and spherical, disk, or lens-shaped, lies against the wall of the irregular hof. It stains normally and vacuoles are generally absent.

The cells near the tip continue to divide mitotically for approximately four days. Owing to the continuance of the nuclear and cell division without the possibility of elongation, the cells become greatly reduced in size (fig. 3, pl. I), and differ from those of the greatly shortened zone of elongation in that the walls are not thrown into folds (entspannt). The difference in the size of the cells that did not divide, as seen in the figure, and those that did, is easily recognized; also the difference in the size of the nuclei [*cf.* HALLBAUER (**10**) p. 15].

In the normal root the cells of the periblem very soon lose the power of division and then simply increase in size. If this increase be prevented, as by imbedding in plaster of Paris, the cell walls continue to grow by intussusception and are, therefore, thrown into folds, or are buckled, or send tube-like projections into the intercellular spaces. PFEFFER (**34**, p. 311, 321) first called attention to this buckling under conditions similar to those described above.

Apparently all growth soon ceases and the cells more than 2.5 mm. from the tip pass prematurely into permanent tissue. Mitotic divisions cease in the very short meristematic zone of the tip but will start anew very soon after the root is freed from the plaster of Paris cast. In this connection it is interesting to note the very rapid growth of the tip if, by chance, the root under high turgor pressure [PFEFFER (**34**), p. 300] pushes off an all too thin layer of the plaster and thus frees its tip. The first and very rapid elongation is due to the straightening of the buckled cell walls. The first apparent cellular changes consist in a slight increase in the quantity of the cytoplasm and the disappearance of the violet threads from the same. Then the nucleus slightly enlarges and regains its regular spherical form. The reticulum remains coarse and narrow meshed for some time, and chromatin in the form of chromocentra collects on the reticulum. The hof and nucleoli appreciably enlarge, and the irregular processes that the hof had sent to the nuclear wall disappear.

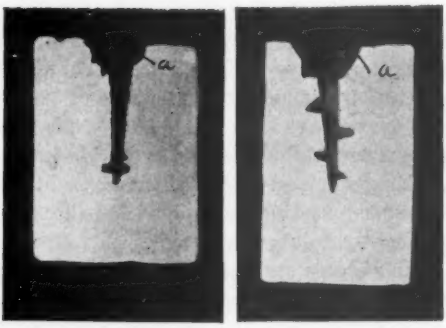
After 10 hours the mitotic figures reappear in increasing numbers. They are, except for size, in every respect normal. The spindle, owing to the irregular arrangement of the cells, is very frequently placed at an angle to the longitudinal axis of the root. The walls, however, are normally formed by the rotation of the phragmoplast.

When in the process of imbedding, air bubbles lodge along the course of the root, the small chambers thus formed are readily filled with tissue as shown in figure 4, plate I. Here the outermost layers of cells are more or less disorganized, while the cells of the first intact row are distinctly elongated in a direction perpendicular to the long axis of the root. This elongation becomes less marked in each successive row, and ceases in the third to the fifth row inclusive, as shown in figure 4, plate I. This growth—plastic growth—is the same as that responsible for filling in the intercellular spaces. Nuclear and cell divisions never occur in connection with the filling in of small chambers.

B. THE GROWTH OF ROOTS IN PLASTER MOLDS

The object of this series of experiments was to determine the manner in which lateral chambers in a mold, of various sizes and at different levels from the tip, were filled in by growth.

The molds were made as follows: Large flat plates of plaster of Paris 1 cm. in thickness were cast and then cut into blocks 3 x 2 cm. Two such blocks were placed with their smooth faces in contact and securely wired. By means of a steel instrument of approximately the same diameter as the root, a passage 2 cm. deep was so bored that one-half appeared in each section of the mold. The mouth of the passage was then enlarged as shown at *a*, figs. 1 and 2. The two halves of the mold were then separated and corresponding lateral chambers cut in the respective halves (figs. 1 and 2).



FIGS. 1 and 2. Molds of plaster of Paris for study of root growth.

The halves of the mold were then brought together again, reinforced by a piece of wood the size of the mold, and the whole securely wired together. A root about 2.5 cm. long was placed in the passage provided, and the widened mouth filled with plaster of Paris. The plaster of Paris served to keep the root in a fixed position and prevented it from backing out when the tip met with resistance. The heavy cotyledons were held in place by a pin thrust through them and into one of the blocks of wood. The seedlings thus prepared were immersed in tap water nearly up to the cotyledons. By the use of molds of this character, the roots may be made to assume almost any conceivable form, fig. 3.

The extent and nature of the growth by which the chambers of the mold are filled, vary with the form of the chamber and the distance it is placed from the tip of the root. A root secured in a mold as illustrated in fig. 2, soon reaches the end of the passage and is effectively hindered from further growth in length. For a number of days, approximately five, nuclear and cell division continue and form cells which in size are very much below the normal for the zone. While this is going on, the lateral chambers of the mold are gradually being filled with tissue by a localized

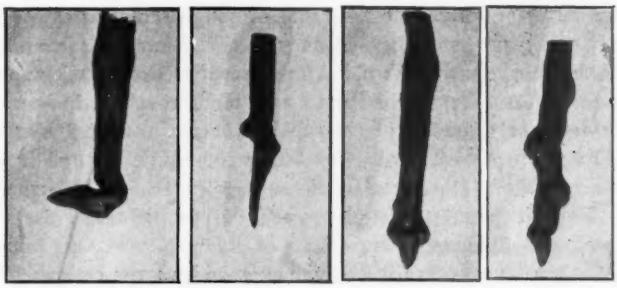


FIG. 3. Roots grown in molds similar to those of figs. 1 and 2. Outgrowths into lateral chambers shown in B, C, and D.

growth in thickness (fig. 3). This growth, by which ultimately an entire cavity is filled is distinctly of two kinds, the mode being determined by the distance of the cavity from the tip. Before entering upon a description of these two kinds of growth, it is essential that we have clearly in mind the nature of the cells, as described for root-tips imbedded in plaster of Paris blocks. The meristematic cells form a very short region of the tip, and the tracheal elements (permanent tissue) have moved acropetal to within 2 mm. of the tip. At this level, the periblem cells have lost the power of nuclear and cell division but still retain the power of growth as demonstrated by the folding or buckling of the walls. A lateral cavity situated in this region will, therefore, be filled by the elongation, laterally, of the cells of the periblem and a gliding of the cells past one another towards the area of least pressure (figs. 4 and 5, pl. I). The process may be compared to the filling of a mold with some plastic mass, and the term "plastic growth" may appropriately be applied [PFEFFER (**34**) p. 268].

In the region in which the tracheal elements have developed, a growth in thickness by nuclear and cell division very early takes place in the pericycle. This is demonstrated most readily by imbedding the tip of a root for only a few millimeters in plaster of Paris, thus preventing growth in length without checking growth in thickness. Such a root will show in a few days a marked increase in thickness on a level with the developed tracheal elements, but this gradually diminishes toward the older regions of the root. A microscopic examination will show this growth in thickness to be due to nuclear and cell division in the pericycle. In roots allowed to remain for some time thus fixed, a change in the rate and position of the thickening will take place. The reason for this becomes obvious when roots (fig. 3 D) grown in molds as illustrated in figure 2, are microscopically

examined. It is of interest to note that although the cavities are of equal size, the lateral growths of the root vary in size, decreasing in either direction from the third above the tip. A microscopical examination shows very clearly that the lateral growths from 1 to 2 mm. from the tip are produced by the enlargement laterally of the cells of the periblem, *i.e.*, by "plastic" and "gliding" growth. The chambers near the tip, if limited in size, are rapidly filled; those at a greater distance from the tip fill more slowly because of the different mode of growth. Here the filling in is due to nuclear and cell division taking place in the pericycle and pushing the tissue of the periblem before it. If the chamber is large enough, the tissue of the periblem is followed by the new tissue arising from this local and premature growth in thickness within the pericycle. The cell division in the pericycle will continue for some time after the chamber has been filled, but ceases when the already very narrow cells have been further reduced in size by cell division without the concomitant cell-stretching.

In contrast to the "plastic growth" earlier described, the growth just discussed may be designated as "correlation growth," [PFEFFER (**34**); HERING (**12**)] that is, a growth by nuclear and cell division that is directly related to and initiated through the interruption of the growth at the tip. At first sight, this correlation growth would seem to be nothing more than the normal growth in thickness. That this is not the case is readily seen by reference to figure 3 D. In this root the fourth lateral outgrowth, in a region in which the growth in thickness would normally be more active because the fundaments are older and farther developed, is, nevertheless, considerably smaller than the second or third from the tip.

The cytoplasm, nucleus, nucleolus, and hof present the characteristics already described under A.

C. The growth of roots under lateral pressure

In the present section it is the intention to follow the method of growth, and, more especially, to determine the position of the mitotic spindle in roots of *Vicia faba* placed under strong lateral pressure.

KNY was the first experimentally to follow a modification of growth in plant tissue as determined by lateral pressure. "Sie geben für das Pflanzenreich den ersten experimentellen Nachweis, dass es möglich ist, die Orientirung der Kernfigur und damit die der Theilungswand dadurch zu bestimmen, das man durch Zug bezw. Druck dem vorhergehenden intensivsten Wachsthum eine bestimmte Richtung willkürlich aufnöthigt" [KNY (**19**), p. 390]. It must be here stated that KNY reached the above conclusion from a study of the position of the cell walls; the orientation of the

mitotic figure was not observed. NĚMEC (**30**, p. 214) repeated the experiments of KNY and paid particular attention to the position of the mitotic figure. He confirms KNY's results in so far as the orientation of the cell wall is concerned, and adds that the pressure experimentally applied, modifies the periplast (p. 242) and through it determines the orientation of the mitotic spindle, and, consequently, the cell wall. He says, "Ich wiederholte einige Versuche, die in dieser Beziehung von KNY ausgeführt wurden (mit Kartoffeln, Wurzeln von Helianthus annuus, Pisum, und Vicia) und fand, dass durch Zug oder Druck die bipolare hyaline Spindelanlage oder wenigstens die Achse der achromatischen Fäserchen orientirt wird. Die Richtungen dieser Achsen stimmen aber mit den Richtungen der geringsten, durch Zug oder Druck eines homogenen festen Körper inducirten Elasticität überein." [NĚMEC (**31**), p. 247]. From the above and also from his discussion on page 242, it is apparent that NĚMEC assumes that the orientation of the spindle in the cells of roots growing under lateral pressure is due to a modification of the form of the "periplast." The periplast with its fluid content is spherical when in complete equilibrium, and becomes elliptical through lateral pressure acting on the cytoplasm. From this one may infer that NĚMEC supposed the cytoplast to be equipotential, that is, isotropic, and that the change in the form of the periplast is due to a change in the cytoplasm in consequence of which it fails to transmit pressure as does a liquid.

In a second contribution KNY again takes up the influence of pressure on cell division and, so far as pertains to his study of the root-tip of *Vicia faba,* still holds to his former view, though he modifies it as follows: "Es zeigt sich hier, wie auch an anderen Stellen des Wurzelquerschnittes, dass die Druckkräfte den durch Erblichkeit vorgezeichneten Zelltheilungsvorgängen bis zu einem gewissen Grade entgegenwirken, sie aber nicht vollstandig unterdrücken" [KNY (**20**) p. 77].

The method used for placing the root under lateral pressure is a modification of the one used by KNY in his earlier work [HOTTES (**16**), p. 41]. Two pieces of plate glass 12.5 cm. square are held together by wooden strips in such a manner that while they meet along their lower edges there is a distance of about 3 mm. between the upper edges. This distance is maintained by the insertion of small cork plugs (fig. 4). The width of the wedge formed by the two plates could be readily varied by simply sliding the wooden strips up or down. Between the two wooden strips on one side was placed a rather strong nickeled steel spring. This gives a constant pressure upon the growing tip.

The seedling was placed in position by carefully shoving the root beween the converging plates until its tip met with a considerable resistance. The

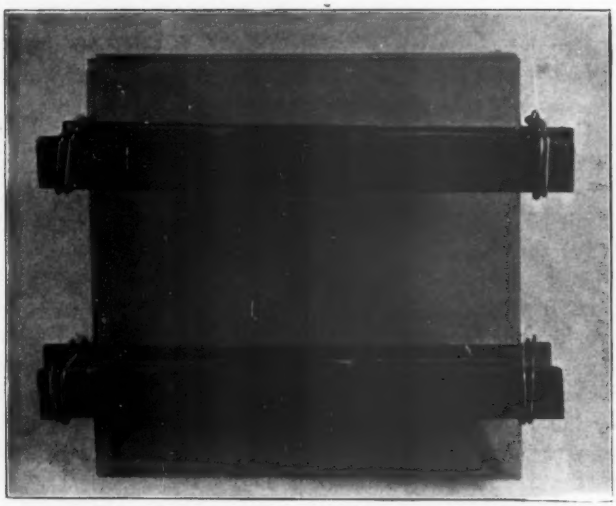


FIG. 4. Glass plate apparatus for producing lateral pressure upon growing roots.

whole was then placed in a dish of tap water reaching nearly to the cotyledons. The apparatus was slightly inclined until the root-tip had by growth wedged itself between the glass plates, when it was placed in a vertical position. Curvature of the tip was by this greatly reduced. When the root had firmly established itself, the wooden bars were moved upward sufficiently to slightly compress the spring. In the wider portion of the wedge, the root grew quite rapidly, but when it reached the portion where the plates were only 0.5 mm. apart, growth in length was greatly retarded and soon ceased. With this retardation or cessation of growth in length, a growth in thickness, parallel to the plates would very soon manifest itself. This latter growth reached its maximum about 3 mm. from the tip, and then decreased in both directions, though the more rapidly toward the tip. This zone of maximum growth agrees perfectly with the growth in thickness described under A and B as "correlation growth."

For reasons similar to those stated under A, it was found necessary to kill and partially fix the root-tips before freeing them from the glass wedge.

Cross-sections at different levels of root-tips grown as above described, agree with the results reported by KNY and NĚMEC in so far as size relations and number of cells are concerned. In several cases the difference in the number of cells parallel and perpendicular to the glass plates was considerably larger than that given by these investigators. Although the number of cells parallel and perpendicular to the glass plates of the wedge is essen-

tially that reported by KNY and NĚMEC, I am convinced that this difference, as also the orientation of the cell walls and mitotic figure, can be accounted for by the forms of growth discussed under A and B.

When the root growing into the ever-narrowing wedge finally reaches its limit of growth, the cells of the tip and of the periblem perpendicular to the glass plates show essentially the same characters as described under A. The cells of the tip when further advancement of the root-tip is mechanically prevented, continue to divide mitotically but fail to elongate in the normal axial direction. A high turgor pressure is developed in such cells, and, in consequence of it, the cell is deformed by sending processes into the intercellular spaces. On the free sides, that is, the sides opposite the pressure, elongation is more extensive and the cells more regular in size and shape. A cross-section of such root-tips frequently shows distinct evidence of a gliding growth. The cells in the diameter perpendicular to the glass plates are soon mechanically prevented from farther elongation; those in the diameter parallel to the glass plates are free to elongate in that direction. In the angles formed between these cell complexes, the lines of a gliding growth are very apparent (fig. 6, pl. I). By careful comparison of the cross-sections of a large number of roots, I find that the mitotic figures, so long as cell division in the tip and the periblem continue, are normally oriented. The difference in the number of the cells on the sides under pressure as compared with those not under pressure is not due to the orientation of the spindle through pressure. From a study of longitudinal sections of roots treated as above, it is very evident that the cause must be attributed, for the greater part, to plastic and gliding growth (fig. 7, pl. I). In addition to this, correlation growth brought on by the cessation of division in the cells that are mechanically prevented from enlarging and the acropetal movement of the permanent tissue plays an interesting and important part. Under A and B it was shown that cell division in both the tip and the pericycle, ceased when the cell was reduced in size by nuclear and cell divisions without the concomitant stretching.

In roots subjected to lateral pressure, a growth in thickness very soon starts. This shows the same peculiarity as that already described in sections A and B, being most rapid at a point a little back of the end of the tracheal elements. This growth, "correlation growth," is due to the multiplication of the cells of the pericycle, perpendicular to the long axis of the root, and is induced by the retardation or cessation of the growth of the tip. The normal growth in thickness, starting later, will eventually overtake that described as correlation growth. In both these modes of growth in thickness the mitotic spindles are normally oriented, that is, perpendicular to the long axis of the root. On the sides of the root not subjected to

pressure nuclear division and growth continue; while on the sides subjected to pressure the nuclear and cell divisions cease when the cells reach a minimum size.

The most interesting and conclusive evidence in support of the above is offered by the study of longitudinal sections of a root grown under pressure. If longitudinal sections perpendicular to the glass plates be made to the center of the root, and the root reimbedded and cut in a plane parallel to the glass plates, the cells in the pericycle will appear respectively as represented in figs. 8 and 9, pl. I. In 9, the nuclear and cell divisions have continued until a large number of rather small and closely packed cells have been produced. The mitotic figures, as represented, are normal in position, and at this time, because of the few cells of even average size, very few in number. Soon, all divisions in this plane will cease. Cell division and growth, however, will continue in a direction parallel to the glass plates.

The mitotic figures in every phase appear normal, and but for occasional exceptions due to irregularities of the cells on account of varying space-accommodation, are placed perpendicular and not parallel to the glass plates. In no case examined could there be found any indication of an effect on the early mitotic phases such as NĚMEC describes (**31** p. 246).

The cells of roots treated as described in sections A, B, and C show many points of similarity. By the methods of experimentation used, the roots in each case were mechanically prevented from growth in length. This inhibition affected both the internal structure of the cell, and the manner and rate of growth in thickness of the root.

The cytoplasm of the tip cells is rapidly reduced in quantity, though its structure is not materially changed. In the cells of the formative region, it has been reduced from one-half to two-thirds its normal extent, and peculiar heavy violet threads or concentric circles are quite common.

The cells of the periblem lose their cytoplasm far more rapidly than do the cells of the plerome or the initial cells of the tip. This loss of cytoplasm, however, does not affect the turgor of the cells as has been shown [PFEFFER (**34**)], and as will appear in a subsequent discussion. In the cells in which the turgor has been greatly increased large numbers of spherical bodies are usually found.

The nucleus of the formative region is somewhat smaller than that in normal cells of similar position and size. The reticulum is very dense, owing to its fine meshes and apparently abundant chromatin. The nucleolus is greatly reduced in size, varying from 1/3 to 1/15 the diameter of the nucleolus in similar cells of the normal root. The hof is absent or greatly reduced in size, and frequently sends an irregular canal to the

nuclear membrane. At the point where this canal meets the nuclear membrane the latter frequently shows a papilla-like protrusion.

Nuclear and cell divisions continue for some time in the formative region even when the cells are mechanically prevented from further growth (fig. 3, pl. I). The size of the nuclei in these newly formed cells is distinctly smaller than in the contiguous larger cells. This with the reduction in quantity of the cytoplasm already stated are of interest in connection with the "nucleus-plasma-relation" of HERTWIG (**13**, p. 4). The nuclear and cell divisions soon cease, and the metabolic nucleus remains apparently unchanged for a period of 30 days. Up to and including this period, the roots begin at once to elongate when freed from the mechanical hindrance. This elongation is due to the high turgor pressure in cells with buckled walls. Nuclear and cell divisions occur in from 5 to 24 hours. The division of the nucleus is always preceded by a slight increase in the cytoplasm. The mitotic figure is normal, but undersize.

All the mitotic figures both in the formative region of the tip and in the pericycle are normal, except for size. In no case was evidence found in support of the aggregate condition of the cytoplasm, which by transmitting pressure unlike liquids, exerts an orienting influence on the mitotic figure through a deformation of the periplast [NĚMEC (**31**), p. 226]. The orientation of the mature spindle is normal, and the difference in the number of cells, etc., parallel and perpendicular to the plane of pressure [*cf.* HOTTES (**16**) p. 43] is due to plastic and gliding and correlation growth [KÖHLER (**21**)].

In a root whose tip is prevented from further growth, the tracheal tissue moves acropetal to within 1 or 2 mm. of the tip, and a correlation growth in the pericycle very soon starts.

When the cells of the periblem have lost the power of nuclear division, they are still in a stage of active growth.

In roots subjected to lateral pressure between two glass plates, active cell growth in the periblem continues on the free sides. This is exactly comparable to the filling of the cavities in plaster of Paris molds as described under B.

If further growth of the tip of the root is mechanically prevented by the converging glass plates and cell division has ceased, the walls of the cells continue to elongate by intussusception and are thrown into folds, or buckle, as shown in figure 4, pl. I. This buckling of the membrane takes place while a very high turgor is maintained in the cells [PFEFFER (**34**), p. 296], and suggests further work on the relation between turgor and growth by wall-stretching [DAVENPORT (**4**), p. 73]. By this process of plastic growth the regular arrangement of the cells becomes disturbed to a greater or less

degree and a process of gliding growth ensues as at *a*, fig. 7, pl. I. This gliding growth, so manifest in longitudinal section, accounts for the following statements by KNY: "Die Rindenzellen greifen unregelmässig zwischen einander ein" (**20,** p. 77). This growth, however, is of relatively small interest as compared with that due to nuclear and cell division in the pericycle. Growth in thickness normally begins in the older and proceeds to the younger regions of the root. When growth in length is prevented, a growth in thickness in the younger regions lying from 2 to 5 mm. back of the tip begins. This produces a swelling, which falls off abruptly toward the tip and more gradually in the opposite direction. Such an abrupt and local growth in thickness was obtained in roots of *Vicia* many times and in different ways. In fact, any agent that destroys or inhibits growth at the tip, is likely to produce this local thickening through correlation growth. Figure 10, pl. I, represents a root of *Vicia* that was grown in moist air and dipped in cold water for an instant every day. SACHS (**39,** p. 801) produced similar roots by the same method. He further observed a similar thickening in a root of *Monstera*, which, while growing along horizontally, struck with its growing tip a rough wall. HOFMEISTER (**15,** p. 161) before SACHS, had observed a similar thickening when roots of *Zea mays* grew against the bottom of the flower pot. Both HOFMEISTER and SACHS at first attributed this thickening to a telescoping of the tender cells when the tip is mechanically stopped and growth in the regions behind it continues. When SACHS obtained similar results on roots in moist air, he gave up this idea without attempting an explanation.

From the above it is apparent that there exists a definite relation between the retardation or inhibition of the growth of the tip and this peculiar mode of growth in thickness. Further, by alternately recurring conditions, now favorable, now unfavorable to growth in length, a more or less moniliform root may be produced (fig. 10, pl. I). It is apparent, then, that this form of growth is due to stimulation, and PFEFFER's term, "correlation growth" may be applied to it.

Anatomical and cytological studies show conclusively that this mode of growth is due to premature nuclear and cell divisions in the pericycle induced through stimulation. The orientation of the spindle, and consequently the position of the cell wall, is not determined as KNY, NĚMEC, and GIESENHAGEN (**9,** p. 45) maintain. The tissue differentiation within the root-tip whose growth is mechanically or otherwise retarded or inhibited, determines through correlation the character of the subsequent growth, and, consequently, the orientation of the mitotic spindle. This premature growth in thickness in the region near the tip starts in roots imbedded in

plaster of Paris, or growing between glass plates, but soon ceases with the reduction in the size of the cells, and is easily overlooked. It can be readily detected if chambers are provided in the region of the root immediately behind the tip, as at the second and third lateral growths from the tips in figure 3 D. In roots subjected to lateral pressure, as described under C, few nuclear and cell divisions occur perpendicular to the plates because of the rapid decrease in the size of the cells. In such as do occur, the orientation of the mitotic spindle is normal, that is, the axis of the figure coincides with the radius of the root. On the sides not under pressure this growth in thickness continues and forms a considerable tissue. The manner of growth is not readily followed in cross-sections, as KNY and NĚMEC evidently studied, but in longitudinal sections prepared as described under C, and as represented by figures 8 and 9, pl. I, is easily apparent.

What has been said of the spindle in "correlation growth" holds equally well for the normal growth in thickness further removed from the tip. The statement of KNY, "Bei Zelltheilungen suchen sich die Scheidewände in der Richtung des Druckes und senkrecht zur Richtung des Zuges zu stellen" (**20,** p. 96), does not agree with the observations here recorded on *Vicia.*

Summary

In roots of *Vicia faba* whose tip is mechanically inhibited from further elongation, nuclear and cell division continue for approximately five days.

Nuclear and cell division cease when the cell reaches a minimum size.

The turgor in such cells may rise many fold above that of normal cells of similar age and position.

Nuclear and cell division in cells of minimum size may again recur when the inhibiting agent is removed, and the interval following the inhibition has not been unduly prolonged.

Turgor, perhaps, more than minimum size, is the inhibitor of nuclear division.

The inhibition of nuclear and cell division and of elongation promotes internal tissue differentiation.

The tracheal tissue may move acropetal to within 2 mm. of the tip.

The premature internal differentiation starts nuclear and cell division in the pericycle and leads to a growth in thickness induced through correlation.

The mitotic figure in every instance is normal in form and orientation.

In cross sections of roots under lateral pressure the number of cells parallel and perpendicular to the line of pressure is due to plastic and gliding and correlation growths.

The radially placed spindle and the resulting cell walls are identically formed and oriented in the earliest stages of correlation growth induced by the inhibition of longitudinal growth.

In the later phases nuclear and cell division cease on the sides under pressure and continue on the free sides.

This leads to a greater number of cells on the free sides and the orientation of the cell wall apparently in accordance with the views of KNY and NĚMEC.

DEPARTMENT OF BOTANY,
THE UNIVERSITY OF ILLINOIS.

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EXPLANATION OF PLATE

FIG. 1. Cells from imbedded root, showing characteristic cytoplasmic features. Note concentric ring structures surrounding a small dense body.

FIG. 2. Nucleus with amoeboid appearance, the nuclear membrane raised in the form of a blister where a branch of the modified hof meets the nuclear membrane.

FIG. 3. Cells reduced in size by continued nuclear and cell division, without elongation. Note the smaller nuclei in the recently formed smaller cells.

FIG. 4. Cells with walls thrown into folds by pressure. The buckling of the walls occurs along with high turgor in the cells.

FIG. 5. Growth behavior of cells in "plastic" growth. Periblem cells elongate laterally, gliding past one another towards the area of least pressure.

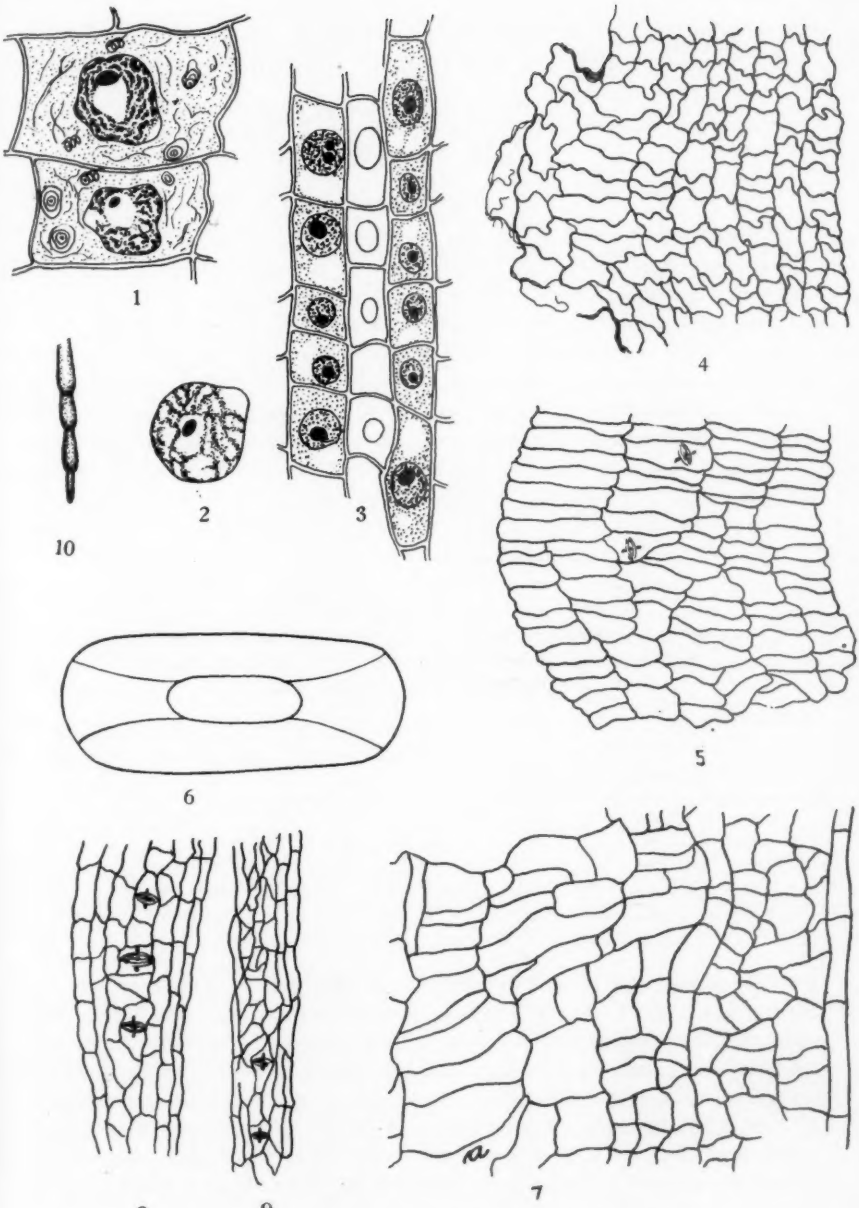
FIG. 6. Cells showing lines of gliding growth, in roots under lateral pressure.

FIG. 7. Gliding growth of cells, under pressure, as at *a*.

FIG. 8. Longitudinal section of root, showing manner of growth of root cells under pressure.

FIG. 9. Longitudinal section of root, in which nuclear and cell divisions have continued until a large number of closely packed cells have been produced. Mitotic figures normal in position but few in number.

FIG. 10. Moniliform root of *Vicia faba*, grown in moist air, and dipped into cold water for an instant every day.



HOTTES—STUDIES IN EXPERIMENTAL CYTOLOGY

THE INFLUENCE OF BORON ON THE CHEMICAL COMPOSITION AND GROWTH OF THE TOMATO PLANT

EARL S. JOHNSTON AND W. H. DORE

(WITH NINE FIGURES)

Introduction

An investigation was undertaken by one of the writers for the purpose of determining the minimum potassium requirements of the tomato plant. It was planned to use water cultures in these studies. Although the solutions contained the so-called essential elements for normal plant growth it was soon discovered that the plants failed to grow. Since attention had been recently called to the importance of boron and manganese it was thought that a possible solution to the problem lay in adding these elements to the culture medium and a special experiment was conducted to test their effects on the growth of the tomato. Manganese (1.0 ppm.) was added to the nutrient solution as manganese sulphate and boron (0.55 ppm.) as boric acid. The four groups noted in table I consisted of nine cultures, each containing a single plant in a two-quart Mason jar.

TABLE I

DATA SHOWING AVERAGE HEIGHT AND DRY WEIGHT OF TOMATO PLANTS GROWN IN BORON DEFICIENT SOLUTIONS AND IN SOLUTIONS WITH 0.55 PPM. BORON AND WITH 1.0 PPM. MANGANESE

GROUP	SOLUTION	HEIGHT	DRY WEIGHT		
			TOPS	ROOTS	TOTAL
		cm.	*gm.*	*gm.*	*gm.*
A	No B and Mn	24.4	3.0	0.3	3.3
B	B and Mn added	55.2	6.4	1.5	7.9
C	Mn added	24.3	3.0	0.3	3.3
D	B added	55.2	5.4	1.2	6.6

An inspection of this table at once shows that the plants of cultures containing boron are far more normal than the others. It cannot, however, be concluded that manganese is not essential. Although chemicals of a good grade were used in these experiments there is no assurance that they were completely free from manganese and boron. Nevertheless, the experiment does show that the tomato plant requires an appreciable amount of boron over and above that present as impurities in the solutions used. Figure 1 shows very clearly the differences in growth of two representative

FIG. 1. Photograph showing boron deficient tomato plant (left) and one grown in a similar solution to which 0.55 ppm. boron as boric acid had been added (right).

plants of this experiment, one (left) from a culture deficient in boron, the other (right) from a similar solution to which boron (0.55 ppm.) as boric acid had been added.

The growth responses of the tomato to boron were so marked and interesting that a more detailed study was undertaken. The necessity of boron to the growth of other plants has been pointed out by other investigators within the last few years. The earlier workers with boron concerned themselves with detecting its presence in various plants. The amounts found as boric acid varied considerably, sometimes running as high as one per cent. or slightly above. The presence of boron *per se* in the plant is, however, no indication that it is essential for growth. The natural supposition would be that its presence is a detriment rather than an asset, since very small amounts are known to be exceedingly toxic. For reviews of the literature and descriptions of experiments indicating the necessity of boron for normal plant growth and development the reader is referred to BRENCHLEY (**2**), BRENCHLEY and WARINGTON (**3**), SOMMER and LIPMAN (**14**), SWANBACK (**15**), and WARINGTON (**16**). In a preliminary paper JOHNSTON and DORE (**8**) called attention to the necessity of boron for growth of the tomato plant.

Experimentation

The experiments described in this paper were carried out in the Division of Plant Nutrition at the University of California and in the Department of Plant Physiology at the University of Maryland. The variety of tomato used at California was the Santa Clara Canner, and at Maryland this same variety and Marglobe were used. The seeds were germinated between layers of moist filter-paper. When the roots were 2 to 10 mm. long the young plants were transferred to a germination net similar to that described by JOHNSTON (7). After the seedlings had reached approximately 2 to 3 cm. in length they were transferred to the culture solutions. Each culture consisted of a single plant supported by means of a little cotton in a paraffined flat cork stopper which fitted into a two-quart Mason jar containing the nutrient solution. The jars were wrapped with heavy paper to exclude most of the light from the roots.

The general nutrient solutions used in the California experiments were made up from the following salts: calcium nitrate, magnesium sulphate, magnesium phosphate (primary and secondary), potassium sulphate, manganese sulphate and ferric tartrate. The approximate calculated concentrations of the usual ions in this general nutrient solution expressed as parts per million and milliequivalents were:

	ppm.	milliequivalents		ppm.	milliequivalents
Ca	200	10.0	NO_3	620	10.0
Mg	60	4.9	SO_4	290	6.0
K	78	2.0	PO_4	74	2.3
Fe (enough to keep plants green)			Mn	1	0.0364

In the Maryland experiments the general nutrient solution was made up from salts specially prepared by J. T. Baker Chemical Co. for the Committee on Salt Requirements of the National Research Council, the manganese sulphate and ferric tartrate were of equally high grade but obtained from other sources. The Maryland solutions of slightly different composition had the following partial volume molecular concentrations:

$Ca(NO_3)_2$	0.005
$MgSO_4$	0.002
KH_2PO_4	0.002
$MnSO_4$	0.0000178

To these general nutrient solutions ferric tartrate (0.5 per cent. solution) was added at the rate of a cubic centimeter per liter per day while the plants were young. After the roots were well developed iron was added less frequently. Boron was added as boric acid to the cultures so designated. Expressed as parts per million and milli-equivalents the ions of the

basic solution used in the Maryland experiments had the following approximate values:

	ppm.	milliequivalents		ppm.	milliequivalents
Ca	200	10.0	NO_3	620	10.0
Mg	49	4.0	SO_4	194	4.0
K	78	2.0	PO_4	190	6.0
Fe (enough to keep plants green)			Mn	1	0.0364

A second preliminary experiment was conducted to determine an approximate range of tolerance of the tomato plant toward boron. Four sets of cultures were set up on October 29, 1926. Each set consisted of 10 two-quart culture jars with a single plant per jar. To the general nutrient solutions boron was added at the following rates:

Group A	0.000 ppm.
Group B	0.055 ppm.
Group C	0.550 ppm.
Group D	5.500 ppm.

The plants were harvested on December 10. The average length of stem and dry weight per plant as well as the approximate total transpiration are given in table II.

TABLE II

DATA SHOWING AVERAGE HEIGHT AND DRY WEIGHT OF TOMATO PLANTS, TOGETHER WITH THEIR APPROXIMATE TOTAL TRANSPIRATION WHEN GROWN IN SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF BORON

Group	Boron	Stem height	Dry weight			Transpiration
			Tops	Roots	Total	
	ppm.	*cm.*	*gm.*	*gm.*	*gm.*	*cc.*
A	0.000	35.1	2.66	0.22	2.88	952
B	0.055	53.4	3.69	0.62	4.31	1584
C	0.550	54.3	3.63	0.63	4.26	1562
D	5.500	45.8	2.75	0.40	3.15	1152

Weekly measurements of stem height were made, but only the final average for each group is included in the table. While making the height measurements on December 2 it was observed that the stems and petioles of the plants in group *A* were exceedingly brittle. In later experiments it was found that this brittleness was characteristic of tomato plants grown in boron deficient solutions. In fact, the brittleness usually occurred before any visible manifestation of boron deficiency appeared. The brittleness of stems and petioles associated with boron deficiency is best described as similar to the breaking of a piece of cheese and not that characteristic of

turgid tissues, which usually break with a snap. The data in table II bring out quite clearly an approximate range of boron concentration, below and above which growth is inhibited. Plants of group A, to which no boron was added, showed a decided retardation of growth and distinct injuries. Another marked symptom of boron deficiency is the dying of the growing point of the stem. In a short time this tissue becomes blackened and dried, thus terminating stem elongation. The stem height measurements of table II bring this out as well as figure 1. Plants of group D, to which 5.5 ppm. of boron were added likewise showed a retardation of growth. These plants also showed distinct injury which, however, was unlike that occurring in the boron deficient plants. With this amount of boron the leaves died at the margins. A representative leaf from a plant of this group together with a leaf from a healthy plant is shown in figure 2. No marked differ-

FIG. 2. Photograph showing boron injury to the leaf of a tomato plant (left) when grown in a nutrient solution containing 5.5 ppm. boron as boric acid. Normal leaf (right) does not show the dried and dead margins.

ences in growth were noted between the plants of groups B and C so that it might be concluded tentatively that boron in these particular culture solutions was equally good for growth of the tomato plant at concentrations of 0.055 and 0.55 ppm. It must be remembered that while 0.055 ppm. of boron may be sufficient for normal growth under one set of conditions it may be insufficient under others.

Immediately following the second experiment an investigation was started with the object of determining the chemical differences between

normal tomato plants and those grown in boron deficient media. Tomato seeds (Santa Clara Canner) were germinated in a manner already described and on December 15, 1926, the seedlings were set out in the culture jars. Two sets of cultures were used in this experiment, one, group A, without boron, except for traces that might have been present as impurities in the culture solutions, and another, group B, to which boron (0.55 ppm.) was added as boric acid. This experiment, as well as the two preliminary ones, was carried out at Berkeley, California. Each group contained 40 plants, four of which were used in preliminary microscopical tests; the remaining 36 plants of each group were harvested on February 7, 1927. The nutrient solutions were not renewed during the entire growing period. In these experiments the amount of mineral elements contained in the solutions was sufficient for good vegetative growth over the periods studied. During certain seasons in other experiments when transpiration rates were higher it was necessary to add distilled water to replace that lost, but this was not necessary in the present case.

Because of the changes in the various constituents (particularly in the amount and kind of carbohydrates) that are taking place in plant tissues during the day, special care was exercised in harvesting the plants in order that the two groups might be as comparable as possible. Six plants from each group were alternately harvested. Considerable time was consumed in this operation and without a doubt plants harvested in the morning differed considerably from those harvested in late afternoon. For this reason it seems advisable to include a table showing the time of day as well as the character of the sky when each set of six plants from the two groups was harvested. These data are presented in table III.

TABLE III

DATA SHOWING ORDER AND CONDITIONS OF HARVESTING TOMATO PLANTS

GROUP	CULTURE NUMBERS	TIME OF HARVEST		CHARACTER OF SKY
		BEGINNING	ENDING	
A and B	1– 6	9:53 a.m.	10:37 a.m.	Bright and dull
A and B	7–12	11:42 "	12:18 p.m.	Bright
A and B	13–18	2:22 p.m.	2:51 "	Bright
A and B	19–24	3:50 "	4:20 "	Bright
A and B	25–30	5:14 "	5:36 "	Clear sunset
A and B	31–36	7:30 "	7:54 "	Dark

Plants (7 to 30) of group B, which were harvested during the warmest and brightest part of the day, were in a wilted condition. The other plants

of group B were turgid when harvested. None of the plants in group A (those deficient in boron) were severely wilted at any time of the day. Plants numbered 10 and 27 in group A, and 3 and 14 in group B differed somewhat in their forms of growth from the other plants. Such variations are to be expected with a variety like the Santa Clara Canner which is highly heterogenous as noted by LESLEY and ROSA (**9**). Despite these variations in growth, injuries were apparent in all of the boron deficient plants.

Analytical methods

PRELIMINARY PREPARATIONS

Preparation of samples.—The harvesting operations consisted of dissecting the plants into leaves, stems and roots. An attempt was made to pull the expanded leaf tissue from the mid-rib. The petiole and often a small portion of the mid-rib were thus included with the stem tissue. The roots were severed in a plane just above the position where they branched out freely from the base of the stem. Without removing the plants from their culture jars the leaves were severed, weighed and dropped into boiling 95 per cent. alcohol. The stems were then cut from the roots and treated similarly, and finally the roots received the same treatment. The boiling process was continued for approximately 15 minutes after the plant tissues had been introduced. The flasks containing the plants and alcohol were then stoppered with paraffined cork stoppers. On cooling, the softened paraffin hardened, automatically sealing the flasks. The material was thus effectively preserved for analysis.

Separation of the sample into alcohol-soluble and alcohol-insoluble matter.—The contents of each flask were filtered and each filtrate was collected in a 1-liter graduated flask. The undissolved portion was placed in Soxhlet extraction tubes and extracted with 85 per cent. alcohol until colorless. The green solution resulting from the extraction was concentrated and added to the original filtrate. The combined alcoholic solution was then made up to volume.

Determination of the alcohol-insoluble dry matter.—The colorless insoluble residue was placed in tared weighing bottles, dried in an oven at 100° C. and weighed. The difference in weight was recorded as *alcohol-insoluble matter*. The material so obtained was used for the determination of *starch, hemicelluloses, galacturonic acid, lignin-suberin* and *cellulose*.

Determination of alcohol-soluble dry matter.—A 25-cc. aliquot portion was taken from each of the liter flasks and total solids were determined by weighing after exaporating in a tared porcelain dish and drying in an oven at 100° C. From the weight of solids thus obtained, the weight of *alcohol-soluble dry matter* in the liter of solution was calculated by multiplying by 40.

Determination of total dry matter.—The value representing the total dry matter was obtained by adding together the values of the *alcohol-soluble* and *alcohol-insoluble dry matter.* Water was determined by subtracting this sum from the corresponding green weight of the tissue.

Analysis of the alcohol-soluble portion

Preparation for the determination of sugars.—100-cc. portions of the alcoholic solutions were placed in Erlenmeyer flasks and evaporated to dryness on the steam bath with the steam turned low so as to avoid undue heating of the sugars. To remove chlorophyll and other substances preparatory to determining the sugars, the dried residue was digested three times with benzene on the steam bath, the benzene solution being poured through a folded filter. The combined benzene filtrates were evaporated in a tared beaker, dried, weighed and recorded as *benzene-soluble matter.*

One portion of the benzene-insoluble residue was in the original flask and another on the folded filter. Both were heated over the radiator to expel adhering benzene. 15 cc. of hot water were next added to the flask and, after a few minutes, when the sugars had dissolved, the solution was poured through the filter-paper dissolving the rest of the residue. Flask and filter were washed with a few cubic centimeters of hot water collecting the solution in a 110-cc. flask.

The filtrate was cooled to room temperature and basic lead acetate added drop by drop until no further precipitation occurred. The excess lead was removed by adding saturated sodium oxalate solution in slight excess. The solution was then made up to 110 cc. and unless the analysis was to be completed at once about 1 cc. of toluol was added to the solution as a preservative.

Reducing sugars.—Reducing sugars were determined on 25-cc. aliquot portions of the prepared solution by a slight modification of the Munson and Walker method, (C. A. Browne, Handbook of Sugar Analysis, p. 426, New York, John Wiley and Sons, 1912.) The precipitated cuprous oxide was collected on tared asbestos Gooch crucibles and washed with water in the usual manner, but instead of drying and weighing as cuprous oxide, the precipitates were ignited in a muffle furnace to cupric oxide and weighed as such. The weight of cupric oxide was multiplied by the factor 0.9 to give the weight of cuprous oxide and the corresponding weight of glucose was then obtained from Munson and Walker's tables.

Reducing sugars after inversion.—55 cc. of the prepared solution were placed in a 110-cc. flask, and 5.5 gm. of solid citric acid added. The mixture was then boiled for 10 minutes. After cooling to room temperature the solution was made alkaline to phenolphthalein by adding NaOH solution

and thereafter dilute acetic acid was added until the red color disappeared. The solution was made up to 110 cc. and reducing sugars determined on 50-cc. portions in the same manner as before.

Sucrose.—Sucrose is obtained by difference between reducing sugars before and after inversion.

Analysis of the alcohol-insoluble portion

Starch.—To 1 gram of the alcohol-insoluble material 100 cc. of water were added, after which the mixture was boiled for 15 minutes to gelatinize the starch. After cooling to room temperature, 0.1 gram of takadiastase and 10 drops of toluol were added. The mixture was placed in the incubator room at 28° C. and left over night. After removing the solution from the incubator room it was boiled for 15 minutes to inactivate the enzyme and then filtered through a Gooch crucible containing a disk of mercerized cotton cloth as the filtering medium. The undissolved residue was saved for the *hemicellulose* determination. The filtrate was transferred to a 200-cc. volumetric flask, clarified with basic lead acetate, deleaded with sodium oxalate solution and made up to volume. The mixture was filtered through a dry filter and 50 cc. of the filtrate were mixed with 5 cc. of concentrated hydrochloric acid and boiled for 2.5 hours under a reflux condenser. After cooling to room temperature the solution was made alkaline with sodium hydroxide solution, then slightly acid with dilute acetic acid. It was then made up to volume and reducing sugars expressed as glucose. Starch was calculated by multiplying by the factor 0.9.

Hemicelluloses.—The residue remaining after the takadiastase digestion was transferred to an Erlenmeyer flask with a measured volume of water. To this were added 8 cc. of 12 per cent. hydrochloric acid and water to make a total volume of 100 cc. The mixture was heated on the hot plate under a reflux condenser for 2.5 hours. The insoluble residue was then filtered off and washed and the filtrate transferred to a 200-cc. volumetric flask. After cooling to room temperature the solution was made alkaline with NaOH, then slightly acid with acetic acid. It was clarified by adding basic lead acetate in slight excess followed by sodium oxalate solution to remove the excess lead. The solution was made up to 200 cc., mixed, filtered through a dry filter, and reducing sugars determined in 50-cc. portions. The results were calculated to the hexosan formula by multiplying the glucose value by 0.9.

Residue after hemicelluloses.—The insoluble residue remaining after the hydrolysis of the hemicelluloses was collected on a Gooch crucible containing a filtering disk of mercerized cotton cloth, dried in the oven at 100° C. and weighed.

Lignin-suberin.—The dried residue after hydrolysis of hemicelluloses was digested for 24 hours in 20 cc. of 72 per cent. sulphuric acid. The mixture was then diluted with 300 cc. of water, heated to boiling, filtered on a Gooch crucible with a cloth filter disk, washed, dried and weighed.

Cellulose by loss.—The material soluble in the 72 per cent. sulphuric acid was regarded as cellulose. The value was obtained by subtracting the *lignin-suberin* value from the *residue after hemicelluloses.*

Galacturonic acid.—Galacturonic acid was determined by DORE'S (5) modification of the Lefevre method. One gram of the alcohol-insoluble dry material was placed in a liter flask with 100 cc. of 12 per cent. hydrochloric acid. The flask was placed in a carbon dioxide absorption train and, after sweeping out the carbon dioxide due to inorganic carbonates and that previously present in the flask by a current of carbon-dioxide-free air, the decarboxylation reaction was carried out by heating the flask in an oil bath at 130° C. The carbon dioxide produced by this reaction was swept out of the flask and absorbed in a weighed Geissler potash bulb, suitable precautions being taken to prevent other reaction products from passing over. The reaction was continued until the Geissler bulb showed constant weight, which usually required 4.5 hours. The weight of carbon dioxide multiplied by 4 gives the weight of hexuronic acid present in the plant tissue. Since the galacturonic acid of pectic substances is the only hexuronic acid definitely known to occur extensively in plant tissues, it is assumed that the hexuronic acid value is identical with the galacturonic acid content. This in turn is a measure of the pectic substances, but since the factor is variable, no attempt has been made to express the results in terms of pectic substance.

Chemical data

The data presented in table IV give in detail the green weights of leaves, stems and roots of individual plants. As is to be expected, considerable variation in growth occurs, but in general the plants in each group were fairly uniform. The average total green weights of the subgroups of group A fluctuate with the time of day at which the samples were obtained. Reference to table III will show this. These average values drop from 41.6 in the first period to 36.9 in the third, then rise to 40.1 in the last. Such variations in green weight are to be expected as the water content of the plants varies with a change in atmospheric conditions throughout the day. The low value of 36.9 in the third period is, however, due to the low value of the leaves of plant numbered 18. Several leaves from this plant had been removed several days previous to the date of harvest and their weights are not included in the table. If the other five values are averaged then 36.9 becomes 38.4. The third, fourth and fifth

subgroups then become practically equal with reference to their total green weights. The subgroup values of group B do not show any regular decrease or increase in the total green weight. With the exception of the fourth period the values are quite uniform. These general observations suggest the possibility that plants of group B are slightly better able to maintain a constant moisture content in spite of the fluctuating condition of their environment which tends to bring about a water deficit of the tissues, while plants of group A seem to lack this ability. Such a general inspection of the data would indicate a somewhat greater state of saturation deficit occurring in the boron deficient plants during the middle of the day than was the case under like conditions in the normal plants grown under similar environmental conditions. This, however, is not the case as will be seen from the discussion of table VI.

The amounts of alcohol-insoluble and alcohol-soluble dry matter in plants grown in boron deficient solutions and in solutions to which boron was added are presented in table V. For convenience this table is divided into three sections, l, s, and r, representing respectively data from leaves, stems and roots. In part V—l it will be noted that no significant difference was found between the average total dry weight per plant in group A and that in group B, the latter being slightly greater, 0.27 gram, or about 14 per cent. In each group the percentage of alcohol-insoluble dry matter is approximately twice that of the alcohol-soluble material. The alcohol-insoluble matter is 7 per cent. less in the boron deficient plants than in the normal plants while the alcohol-soluble dry matter is correspondingly increased. A survey of these values for the plant stems in part V—s shows an increase of approximately 60 per cent. in dry matter of the normal plants over the boron deficient ones, these values being 2.83 and 1.76 grams respectively. While the actual difference between the two groups is significant, the percentage of alcohol-insoluble and alcohol-soluble matter of the total dry weight is the same. Part V—r clearly shows the greater average total dry matter in the roots of group B plants. Here the increase is over 200 per cent., by far the greatest of measurable quantities noted in these experiments. The relative proportion of alcohol insoluble and soluble matter in the two groups is practically the same.

So far as the data of table V go, it appears that no marked difference in chemical composition of roots and stems is to be expected between plants of the boron deficient group and those grown in a medium containing boron. There is, however, indication of a difference in chemical composition of the leaves from plants in these two groups. The leaves of the boron deficient plants contain a higher percentage of alcohol-soluble matter than do the normal leaves. Since many of the metabolically important constituents

TABLE IV

DATA SHOWING GREEN WEIGHTS OF LEAVES, STEMS, ROOTS AND THE TOTAL WEIGHT OF INDIVIDUAL PLANTS GROWN IN BORON DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON (0.55 PPM.) WAS ADDED

No.	GROUP A (BORON DEFICIENT)				GROUP B (BORON—0.55 PPM.)			
	Leaves	Stems	Roots	Total	Leaves	Stems	Roots	Total
1	15.0	24.0	4.3	43.3	15.6	32.5	10.9	59.0
2	12.9	22.4	2.8	38.1	16.2	33.9	14.2	64.3
3	14.6	22.6	3.7	40.9	16.8	31.0	15.2	63.0
4	16.2	20.8	3.0	40.0	13.9	30.3	11.1	55.3
5	15.5	22.6	3.4	41.5	15.3	35.8	11.5	62.6
6	17.8	24.8	3.1	45.7	16.2	32.7	13.6	62.5
Total	92.0	137.2	20.3	249.5	94.0	196.2	76.5	366.7
Average	15.3	22.9	3.4	41.6	15.7	32.7	12.8	61.1
7	14.6	22.6	3.1	40.3	15.3	33.7	12.9	61.9
8	14.9	22.9	3.4	41.2	16.5	31.7	14.2	62.4
9	16.7	22.0	2.9	41.6	16.1	33.0	17.3	66.4
10	14.1	19.9	3.2	37.2	15.4	34.2	12.3	61.9
11	14.1	21.2	2.8	38.1	14.3	31.2	11.9	57.4
12	12.6	21.1	3.4	37.1	13.6	30.6	12.9	57.1
Total	87.0	129.7	18.8	235.5	91.2	194.4	81.5	367.1
Average	14.5	21.6	3.1	39.3	15.2	32.4	13.6	61.2
13	14.5	20.7	3.4	38.6	16.2	34.7	17.1	68.0
14	15.8	18.4	2.8	37.0	18.1	32.4	16.8	67.3
15	14.6	20.6	3.8	39.0	15.2	31.6	13.0	59.8
16	12.4	21.7	3.5	37.6	15.2	30.3	12.7	58.2
17	13.9	22.5	3.4	39.8	16.6	28.8	16.3	61.7
18	6.4	20.0	3.2	29.6	13.3	30.5	10.2	54.0
Total	77.6	123.9	20.1	221.6	94.6	188.3	86.1	369.0
Average	12.9	20.7	3.4	36.9	15.8	31.4	14.4	61.5
19	15.6	23.1	2.8	41.5	16.7	35.0	12.6	64.3
20	12.9	20.6	3.5	37.0	15.9	35.4	13.3	64.6
21	12.6	21.0	3.2	36.8	17.4	41.7	14.1	73.2
22	13.0	19.3	3.3	35.6	15.8	34.3	14.4	64.5
23	14.3	22.7	3.6	40.6	17.3	35.3	14.6	67.2
24	17.4	20.6	3.1	41.1	16.9	36.7	14.8	68.4
Total	85.8	127.3	19.5	232.6	100.0	218.4	83.8	402.2
Average	14.3	21.2	3.3	38.8	16.7	36.4	14.0	67.0
25	15.3	20.3	3.7	39.3	13.8	28.4	9.0	51.2
26	12.6	21.9	3.0	37.5	16.8	34.6	14.1	65.5
27	12.5	21.6	3.1	37.2	16.6	34.1	13.5	64.2
28	15.6	21.6	2.4	39.6	16.9	35.5	14.8	67.2
29	13.4	21.1	3.0	37.5	17.2	35.8	13.7	66.7
30	13.8	21.6	3.7	39.1	16.0	32.3	12.0	60.3
Total	83.2	128.1	18.9	230.2	97.3	200.7	77.1	375.1
Average	13.9	21.4	3.2	38.4	16.2	33.5	12.9	62.5
31	13.6	22.5	3.6	39.7	15.7	39.4	11.6	66.7
32	15.0	22.8	3.8	41.6	15.0	33.7	11.8	60.5
33	11.3	19.8	3.2	34.3	16.9	35.6	12.3	64.8
34	15.4	20.5	3.6	39.5	17.1	33.5	11.4	62.0
35	16.6	23.5	3.3	43.4	14.2	35.0	9.3	58.5
36	16.0	23.2	2.9	42.1	16.3	30.2	9.9	56.4
Total	87.9	132.3	20.4	240.6	95.2	207.4	66.3	368.9
Average	14.7	22.1	3.4	40.1	15.9	34.6	11.1	61.5
Grand total	513.5	778.5	118.0	1410.0	572.3	1205.4	471.3	2249.0
Grand average per plant	14.3	21.6	3.3	39.2	15.9	33.5	13.1	62.5

TABLE V—l

DATA SHOWING AMOUNT OF DRY MATTER IN *leaves* OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

GROUP A (BORON DEFICIENT)	ALCOHOL INSOLUBLE		ALCOHOL SOLUBLE		TOTAL
	gm.	*per cent.*	*gm.*	*per cent.*	*gm.*
1– 6	7.62	64	4.32	36	11.94
7–12	7.22	62	4.41	38	11.63
13–18	6.72	*	*	*	*
19–24	6.96	61	4.39	39	11.35
25–30	6.68	61	4.27	39	10.95
31–36	7.06	61	4.57	39	11.63
Average per plant	1.17	62	0.73	38	1.91
Group B (boron 0.55 ppm.)					
1– 6	8.40	69	3.72	31	12.12
7–12	8.93	70	3.74	30	12.67
13–18	9.11	69	4.12	31	13.23
19–24	9.84	69	4.49	31	14.34
25–30	9.41	70	3.96	30	13.37
31–36	8.82	69	3.96	31	12.78
Average per plant	1.51	69	0.67	31	2.18

* Results not determined because alcohol-soluble material of these leaves was lost from a broken flask.

TABLE V—s

DATA SHOWING AMOUNT OF DRY MATTER IN *stems* OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

GROUP A (BORON DEFICIENT)	ALCOHOL INSOLUBLE		ALCOHOL SOLUBLE		ORIGINALLY ALCOHOL SOLUBLE BUT SUBSEQUENTLY PRECIPITATED		TOTAL
	gm.	*per cent.*	*gm.*	*per cent.*	*gm.*	*per cent.*	*gm.*
1–18	21.32	65.0	11.26	34.4	0.21	0.6	32.79
19–36	19.99	65.7	10.34	34.0	0.08	0.3	30.41
Average per plant	1.15	65.35	0.60	34.2	0.01	0.45	1.76
Group B (boron 0.55 ppm.)							
1–18	32.18	66.4	15.84	32.7	0.45	0.9	48.47
19–36	34.61	64.7	18.41	34.4	0.45	0.8	53.47
Average per plant	1.86	65.5	0.95	33.6	0.02	0.9	2.83

TABLE V—r

DATA SHOWING AMOUNT OF DRY MATTER IN *roots* OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

GROUP A (BORON DEFICIENT)	ALCOHOL INSOLUBLE		ALCOHOL SOLUBLE		TOTAL
	gm.	*per cent.*	*gm.*	*per cent.*	*gm.*
1–18	3.96	81.5	0.90	18.5	4.86
19–36	4.17	81.9	0.92	18.1	5.09
Average per plant	0.23	81.7	0.05	18.3	0.28
Group B (boron 0.55 ppm.)					
1–18	13.29	84.2	2.50	15.8	15.79
19–36	13.41	83.3	2.68	16.7	16.09
Average per plant	0.74	83.8	0.14	16.3	0.88

of plant sap are soluble in alcohol, these results immediately suggest that there are significant differences in the active mobile constituents of the two groups of plants; while the insoluble constituents, which include the framework constituents of the plant, are much alike in the two groups. As will be shown later, this is the situation which actually exists.

In table VI data are presented which show the water content of leaves, stems and roots as related to the green and dry weights of plants grown in boron deficient solutions and in solutions containing boron. The weight measurements are expressed in grams and represent the total weight from 6 plants in the case of leaves and from 18 plants in case of stems and roots. In the discussion of table IV attention was called to an apparent decrease in water content in plants of group A. In table VI such a decrease is apparent for leaves of plants in group A harvested during the warm part of the day namely, plants numbered, 7–12, 19–24 and 25–30. However, if the water content of the leaves of each group is compared with the dry weight values, the ratio of water per unit of dry matter is found to be fairly constant throughout the day. On the other hand, these ratio values for leaves of plants in group B fluctuate quite regularly with the periods of the day during which they were harvested. It must be concluded from the data in this table that leaves of the normal tomato plants (group B) showed a tendency to lose water toward midday, while those in the boron deficient group maintained practically the same water-dry weight ratio values throughout the day. Actual observation of the plants as they were harvested bears this out. The leaves of plants 7 to 30 of group B that were collected during the middle of the day were wilted. Leaves of the other plants of this group, 1 to 6 and 31 to 36, which were collected in early

TABLE VI

DATA SHOWING THE WATER CONTENT OF LEAVES, STEMS AND ROOTS OF PLANTS GROWN IN BORON DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

CULTURES	GROUP A (BORON DEFICIENT)				GROUP B (BORON–0.55 PPM.)			
	GREEN WEIGHT	DRY WEIGHT	WATER	WATER PER UNIT DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	WATER	WATER PER UNIT DRY WEIGHT
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Leaves 1–6	92.0	11.94	80.06	6.71	94.0	12.12	81.88	6.76
7–12	87.0	11.63	75.37	6.48	91.2	12.67	78.53	6.20
13–18	*	*	*	*	94.6	13.23	81.37	6.15
19–24	85.8	11.35	74.45	6.56	100.0	14.34	85.66	5.97
25–30	83.2	10.95	72.25	6.60	97.3	13.37	83.93	6.20
31–36	87.9	11.61	76.29	6.57	95.2	12.78	82.42	6.45
Average per plant	14.5	1.91	12.61	6.57	15.9	2.18	13.72	6.29
Stems 1–18	390.8	32.79	358.01	10.92	578.9	48.47	530.43	10.94
19–36	387.7	30.41	357.29	11.75	626.5	53.47	573.03	10.72
Average per plant	21.6	1.76	19.87	11.33	33.5	2.83	30.65	10.83
Roots 1–18	59.2	4.86	54.34	11.2	244.1	15.79	228.31	14.5
19–36	58.8	5.09	53.71	10.6	227.2	16.09	211.11	13.1
Average per plant	3.28	0.28	3.00	10.9	13.09	0.88	12.21	13.8

* Results not determined because alcohol soluble material of these leaves was lost from a broken flask.

morning and late evening were not wilted. From the work of RENNER (**12**), LIVINGSTON and BROWN (**11**), LIVINGSTON (**10**), JOHNSTON (**6**) and others, such a diminution in water content is to be expected during the day in normal healthy plants. An interesting point to be mentioned is that none of the leaves from plants in group A was severely wilted at any time. On *a priori* reasoning alone it might be concluded that either the water absorbing and conducting tissues of the boron deficient plants were entirely adequate to supply water to the leaves as fast as lost by transpiration, or that the structure of the leaves was such as to greatly retard water loss.

There is but little difference to be noted in the average dry weight of leaves per plant of the two groups. A more striking difference is seen in the dry weights of the stems and roots of the plants of these groups. The average dry weight of stems of the normal plants was approximately 1.6 as great as the stems of the boron deficient plants. The most striking difference between the boron deficient plants and the normal ones occurs in the root development. Roots of the normal plants are practically three times

as great when dry weight is used as the criterion of measurement. Poor root development is apparently a definite characteristic of boron deficient plants since it has been observed in other plants by other investigators.

Table VII presents data showing the composition of alcohol-soluble matter in plants grown in boron deficient solutions and in solutions to which boron was added. These values are expressed as percentages of total dry matter in the leaves, stems and roots respectively. The leaves of the boron deficient plants have in every case a much higher content of reducing sugars and of total sugars than the corresponding normal plants. With but one exception the same relationship holds for their sucrose content. The total sugars in the leaves of the normal plants increase as the day advances to a maximum in the late afternoon. The total sugars in the leaves of the boron deficient plants likewise rise to a maximum in the late afternoon, but unlike the normal plants, the total sugars do not drop off in the early evening hours. In this respect the boron deficient plants show distinctly abnormal behavior, the significance of which will be discussed later.

The stems of the boron deficient plants have lower contents of reducing sugars and total sugars than the stems of the normal plants, the relationship being the reverse of that found in the leaves. The meaning of this will also be discussed later. The relationships in the roots are similar to those in the leaves; the boron deficient roots have much higher contents of reducing sugars, sucrose and total sugars.

Another very striking difference between the plants of the A and B groups exists in the percentages of benzene-soluble matter. This material occurs more abundantly in the leaves of the normal plants and in the stems of the boron-deficient plants. The values for the roots are practically the same. This benzene-soluble matter may include chlorophyll, fats, sterols and lipins. Unfortunately we have not found it practicable as yet to undertake a thorough study of this material.

The analytical data showing the composition of the alcohol-insoluble matter of these plants are given in table VIII. These data are likewise expressed as percentages of total dry matter for each of the plant tissues studied. Perhaps the most striking difference is the greater starch content of all the parts of the boron-deficient plants. The hemicellulose is likewise somewhat greater in the leaves of group A plants, but slightly less when the stems are compared. Galacturonic acid gives somewhat higher values for the leaves of group B plants. It is doubtful if the other values are significant. Cellulose is slightly higher in the leaves and stems of the normal plants.

The analytical data presented in tables VII and VIII have been summarized in table IX. For the leaves, the outstanding differences between

TABLE VII

DATA SHOWING COMPOSITION OF ALCOHOL-SOLUBLE MATTER IN LEAVES, STEMS AND ROOTS OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED. THESE DATA ARE EXPRESSED AS PERCENTAGES OF TOTAL DRY MATTER OF LEAVES, STEMS AND ROOTS RESPECTIVELY

CULTURES	GROUP A (BORON-DEFICIENT)					GROUP B (BORON—0.55 PPM.)				
	ALCOHOL-SOLUBLE MATTER	REDUCING SUGARS (HEXOSES)	SUCROSE	TOTAL SUGARS	BENZENE SOLUBLE MATTER	ALCOHOL-SOLUBLE MATTER	REDUCING SUGARS (HEXOSES)	SUCROSE	TOTAL SUGARS	BENZENE SOLUBLE MATTER
Leaves 1– 6	36	6.84	2.16	9.00	9.36	31	2.79	1.24	4.03	13.64
7–12	38	9.12	0.76	9.88	8.74	30	4.20	0.90	5.10	13.50
13–18	*	*	*	*	*	31	3.72	1.55	5.27	12.40
19–24	39	7.41	5.46	12.87	10.14	31	3.72	2.48	6.20	12.71
25–30	39	9.36	3.51	12.87	10.14	30	4.50	0.60	5.10	12.90
31–36	39	8.19	4.68	12.87	9.75	31	4.03	1.24	5.27	13.02
Average per plant	38	8.18	3.31	11.50	9.63	31	3.83	1.34	5.16	13.03
Stems 1–18	34.4	4.21	3.29	7.50	3.42	32.7	7.62	2.42	10.04	1.57
19–36	34.0	6.43	2.96	9.39	3.35	34.4	9.77	3.37	13.14	1.91
Average per plant	34.2	5.32	3.13	8.45	3.39	33.6	8.70	2.89	11.59	1.74
Roots 1–18	18.5	2.57	1.79	4.36	4.01	15.8	0.95	1.44	2.39	4.65
19–36	18.1	1.90	2.34	4.24	4.13	16.7	1.18	1.64	2.82	4.68
Average per plant	18.3	2.24	2.07	4.30	4.07	16.3	1.07	1.54	2.61	4.67

* Results not determined because alcohol-soluble material of these leaves was lost from a broken flask.

the two groups are their sugar, benzene-soluble matter and starch content. Somewhat similar differences appear in the stems, but the order is reversed for total sugars and benzene-soluble matter. Sugars formed in the leaves are, under normal conditions, transported to the stem. In the boron-

TABLE VIII

DATA SHOWING COMPOSITION OF ALCOHOL-INSOLUBLE MATTER IN LEAVES, STEMS AND ROOTS OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED. THESE DATA ARE EXPRESSED AS PERCENTAGES OF TOTAL DRY MATTER OF LEAVES, STEMS AND ROOTS RESPECTIVELY

CULTURES	GROUP A (BORON-DEFICIENT)						GROUP B (BORON 0.55 PPM.)					
	ALCOHOL-INSOLUBLE MATTER	STARCH	HEMICELLULOSE	GALACTURONIC ACID	LIGNIN SUBERIN	CELLULOSE	ALCOHOL-INSOLUBLE MATTER	STARCH	HEMICELLULOSE	GALACTURONIC ACID	LIGNIN SUBERIN	CELLULOSE
Leaves												
1– 6	64	12.85	3.07	8.32	1.66	11.71	69	6.02	2.10	8.14	1.76	12.49
7–12	62	12.05	2.53	6.82	1.24	9.83	70	7.17	2.27	9.94	1.58	12.95
13–18	62*	11.61	2.38	8.56	1.67	11.93	69	7.67	2.18	10.35	1.45	10.94
19–24	61	11.61	2.44	7.44	1.49	10.52	69	8.89	1.38	10.07	1.38	12.11
25–30	61	11.61	2.39	9.27	1.31	9.30	70	10.81	1.99	7.98	1.96	10.26
31–36	61	12.44	2.51	9.88	1.37	9.18	69	9.88	1.38	10.49	2.07	12.59
Average per plant	62	12.03	2.55	8.38	1.46	10.41	69	8.41	1.88	9.50	1.70	11.89
Stems												
1–18	65.0	5.80	4.78	11.05	6.05	20.57	66.4	1.27	5.74	11.22	6.01	24.60
19–36	65.7	5.04	4.65	11.69	5.55	22.08	64.7	1.63	5.95	11.00	5.08	25.43
Average per plant	65.4	5.42	4.72	11.37	5.80	21.32	65.6	1.45	5.85	11.11	5.55	25.02
Roots												
1–18	81.5	0.65	7.04	12.06	14.30	18.58	84.2	0.34	6.60	9.43	12.71	19.07
19–36	81.9	1.31	6.35	10.97	14.66	18.14	83.3	0.13	6.76	11.00	12.91	18.45
Average per plant	81.7	0.98	6.70	11.52	14.48	18.36	83.8	0.24	6.68	10.22	12.81	18.76

* Results not determined because alcohol-soluble material of these leaves was lost from a broken flask. Average of other values.

deficient plants the sugars accumulated because of the apparent inability of the conducting tissues in these plants to translocate them. These data for the stems show that in the normal plants with uninjured phloem tissue the sugars pass into the stems and being able to continue further in these stems very little is condensed to starch. On the other hand in the stems of the boron-deficient plants with their injured conducting systems, the sugars cannot be moved so rapidly and are condensed to starch.

TABLE IX

SUMMARY OF ANALYTICAL DATA OF TOMATO PLANTS EXPRESSED AS PERCENTAGES OF TOTAL DRY MATTER OF LEAVES, STEMS AND ROOTS RESPECTIVELY

AMOUNT OF BORON ADDED TO NUTRIENT SOLUTION (PPM.)	LEAVES		STEMS		ROOTS	
	A 0.00	B 0.55	A 0.00	B 0.55	A 0.00	B 0.55
Reducing sugars (hexoses)	8.18	3.83	5.32	8.70	2.24	1.07
Sucrose	3.31	1.34	3.13	2.89	2.07	1.54
Total sugars	11.50	5.16	8.45	11.59	4.30	2.61
Benzene soluble matter	9.63	13.03	3.39	1.74	4.07	4.67
Starch	12.03	8.41	5.42	1.45	0.98	0.24
Hemicellulose	2.55	1.88	4.72	5.85	6.70	6.68
Galacturonic acid	8.38	9.50	11.37	11.11	11.52	10.22
Lignin and suberin	1.46	1.70	5.80	5.55	14.48	12.81
Cellulose	10.41	11.89	21.32	25.02	18.36	18.76

A very striking characteristic of the boron-deficient tomato plants is the extreme brittleness of the petioles and mid-ribs. This brittleness is perhaps best described as similar to the breaking of a piece of cheese. It is entirely unlike the breaking of a turgid tissue or stem. Because of this peculiar characteristic, it was suspected that pectic substances were absent, or at least less abundant, in the middle lamella of the boron-deficient plants. Absence of a cementing substance between the cells might be the reason for such brittleness. At the suggestion of Prof. J. H. Priestley, microscopic examinations were made using the customary pectin stains but no distinct differences could be observed between sections from normal and from boron-deficient plants. The galacturonic acid determinations given in table VIII are not significantly different for the two groups, and when these figures are calculated to the basis of alcohol-insoluble dry matter (a more comparable basis, since the fluctuating soluble constituents are eliminated) the difference between the two groups is so slight as to fall within the experimental error of the determination. It is apparent that neither the microchemical observations nor the galacturonic acid determinations support the

theory that brittleness is due to a deficiency of pectic material. A special experiment was therefore carried out for the purpose of obtaining enough leaf material for pectic analysis.

This experiment was made at the University of Maryland. On November 1, 1927, young tomato seedlings were set out in two-quart culture jars. The cultures were divided into three groups, a single plant per culture. All the solutions were similar with the exception of their boron content. To group A no boron was added. Groups B and C contained 0.011 and 0.55 ppm. respectively of boron as boric acid. At the end of six weeks the leaves were cut from the plants at the base of the leaf blade. It is unfortunate that the petioles were not included in the samples, but since the mid-rib shows the same characteristic brittleness as the petioles, not a great deal of additional information would have been obtained by including the petioles. The pectic materials[1] were determined by the general methods described by CONRAD (**4**) and by APPLEMAN and CONRAD (**1**). The data are presented in table X.

TABLE X

DATA SHOWING RELATION OF BORON TO PECTIC MATERIALS IN TOMATO LEAVES EXPRESSED AS PERCENTAGE OF THEIR DRY WEIGHT

AMOUNT OF BORON ADDED TO NUTRIENT SOLUTION (PPM.)	0.00	0.011	0.55
Pectin	0.00	0.00	0.00
Pectic acid and pectates	0.00	0.00	0.00
Protopectin	6.98	6.54	5.85
Reducing sugar	5.42	4.51	4.11
Sucrose	0.22	0.31	0.50
Total sugar	5.64	4.82	4.61

So far as pectin, pectic acid and pectates are concerned no measurable quantities were found in any of the groups, even though the boron deficient plants were characteristically brittle. The leaves of plants supplied with 0.55 ppm. boron showed somewhat less protopectin than the leaves of group A. It must be concluded from the evidence here presented that the brittleness is due to something other than pectic materials. It is interesting to note that the total sugar analyses follow in general the results obtained in the California experiments.

Growth data

The first boron experiment conducted at Maryland was outlined for the purpose of comparing the growth of two different varieties of tomato plants

[1] The analyses in this experiment were made by Dr. C. M. Conrad.

in water cultures of three concentrations of boron, 0.0, 0.011 and 0.55 ppm. Each of the six groups was composed of 20 cultures of one plant each. The experiment covered the period from September 10 to October 22, 1927. The average data per plant of each group are presented in table XI.

TABLE XI

DATA SHOWING WEEKLY HEIGHT (CM.), TOTAL TRANSPIRATION (CC.) AND GREEN AND DRY WEIGHTS (GM.) EXPRESSED AS THE AVERAGE PER PLANT PER EACH GROUP

WEEK ENDING 1927	PLANT GROUPS					
	A	a	B	b	C	c
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
September 17	2.5	2.8	3.1	3.3	3.0	3.3
24	4.2	4.4	6.5	6.7	6.3	6.8
October 1	7.4	5.8	14.3	13.2	14.2	13.7
8	13.0	10.0	28.1	24.6	29.2	26.0
15	17.3	13.3	29.2	27.1	35.8	31.6
22	19.6	17.0	29.3	27.5	41.4	36.5
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Transpiration	380	253	1565	1389	2085	2033
Green weight	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Tops	15.6	11.6	43.8	40.5	50.9	48.6
Roots	2.7	2.3	10.7	10.6	18.9	19.4
Total	18.3	13.9	54.5	51.1	69.8	68.0
Dry weight						
Tops	1.32	0.95	4.20	3.89	4.79	4.29
Roots	0.14	0.11	0.69	0.67	1.35	1.31
Total	1.46	1.06	4.89	4.56	6.14	5.60

Note: A, B, C; Santa Clara Canner; a, b, c, Marglobe.
A, a, No boron added to solutions.
B, b, 0.011 ppm. boron added as boric acid.
C, c, 0.55 ppm. boron added as boric acid.

The first boron-deficiency signs were noted in groups A and a on September 19, only nine days after the seedlings were set out in the culture jars. Four days later the cotyledons and leaves were a distinct purple in color. In all probability this color was due to anthocyan, which is frequently associated with an excess sugar accumulation. Such an excess was found in the California experiments and later to a less degree at Maryland. The indications were that the conducting tissues were either destroyed or never properly developed in these plants.

The height measurements at the end of the first week indicate some injury to plants of groups A and a, and by the end of the second week little

doubt can be held as to the falling off in their rates of stem elongation. In groups A and a the terminal shoot soon died. This brought about a very peculiar form of growth. One, and often several lateral shoots developed in a manner illustrated in figures 3 and 4. The dead terminal shoot is clearly seen in figure 3. Attention is also directed to the swollen condition

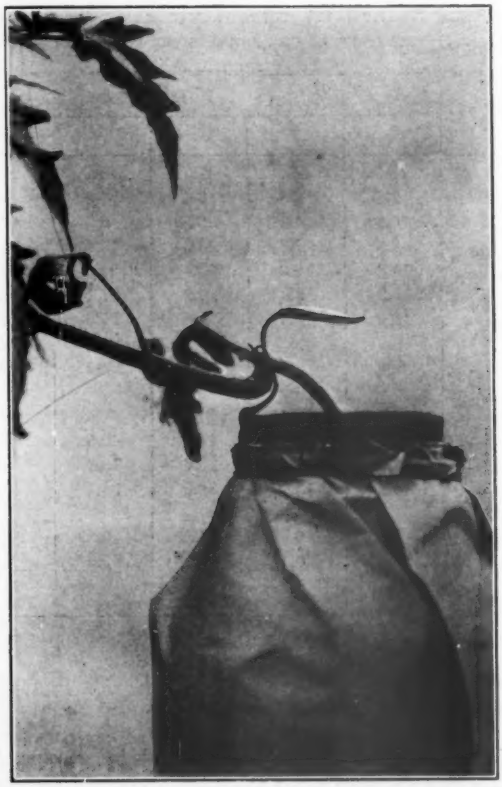


FIG. 3. Photograph showing a boron-deficient tomato plant, with dead terminal shoot and lateral shoot greatly thickened at its base.

of the new shoot at a point just above the place where it joins the main stem. It would appear as if reserve food materials were accumulating at this point because of the inability of the conducting systems to transport them down the main stem.

A comparison of the two varieties shows Santa Clara Canner somewhat superior to Marglobe when stem height, and green and dry weights are used as growth criteria. Each variety was equally susceptible to boron-deficiency injuries and for such studies either one may be used. The data

FIG. 4. Photograph showing a boron-deficient tomato plant in which a number of new shoots have arisen below the dead terminal shoot.

of table XI show another interesting point. The values for groups B and b at the end of the experiment are between those for groups A, a and C, c in every case. Indications are that boron has a quantitative effect. With 0.011 ppm. boron in the nutrient solution, growth could take place at a rate similar to that where 0.55 ppm. was used, but only up to a certain point. From this point the inadequate supply of boron became the limiting factor. This is quite clearly shown in the weekly height measurements. Practically no difference in height existed on October 1 between groups B and C, and b and c. One week later, B and b plants showed signs of retarded growth and by October 22 there was no question as to their inferiority when compared with the plants of groups C and c. This difference in stem heights is shown in figure 5. In a similar experiment carried out a little later in the year under slightly less favorable growing conditions practically the same results were found. Representative plants from the three groups in this experiment (boron added to solutions were 0.0, 0.011,

FIG. 5. Photograph showing tomato plants growing in similar solutions with the exception of the boron concentration. Left, without boron; center, 0.011 ppm. boron; right, 0.55 ppm. boron.

0.55 ppm.) are shown in figure 6. The growth curves of groups A, B, C, for which data appear in table XI are presented in figure 7 and perhaps

FIG. 6. Photograph showing tomato plants growing in similar solutions with the exception of the boron concentration. Left, without boron; center, 0.011 ppm. boron; right, 0.55 ppm. boron.

show to a better advantage the quantitative effect of boron on stem elongation.

Another experiment was carried out with the purpose of growing plants for a period of 6 weeks in a solution supplied with boron, then changing to a boron-deficient solution, and also, to grow other plants in a boron-deficient solution and then change to a solution containing boron. This

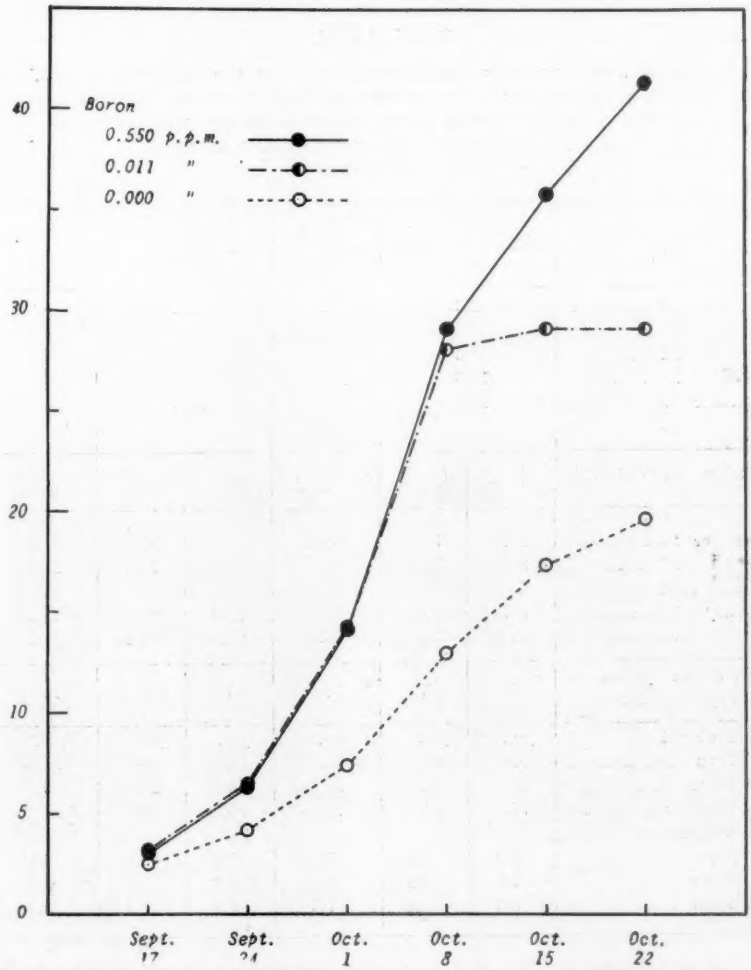


FIG. 7. Average height (cm.) of tomato plants grown in nutrient solutions deficient in boron and in solutions to which 0.011 and 0.55 ppm. boron had been added.

experiment was performed at Maryland with the Marglobe tomato. The plants were grown from November 1 to December 13 without changing the nutrient solutions. They were then grown for four more weeks after the proper changes had been made. The data of this experiment are presented in detail in table XII. At the end of 6 weeks distilled water was added to cultures whose solutions were not changed. All culture jars were of the two quart size with one plant per culture.

TABLE XII

DATA SHOWING AVERAGE HEIGHT (CM.) AND FINAL GREEN AND DRY WEIGHTS (GM.) OF TOMATO PLANTS GROWN IN NUTRIENT SOLUTIONS TO WHICH BORON HAD AND HAD NOT BEEN ADDED AT THE BEGINNING OF THE EXPERIMENT AND AFTER 6 WEEKS. AMOUNT OF BORON ADDED IS SHOWN IN PARENTHESIS AS PPM.

Period ending 1927	(0.55)		(0.55)	(0.0)		(0.0)
	cm.		*cm.*	*cm.*		*cm.*
November 8	2.5		2.4	2.1		2.4
15	4.7		4.4	4.4		4.6
22	7.5		7.0	7.3		7.5
29	13.6		12.4	13.2		13.4
December 6	17.8		16.1	17.5		17.9
13	24.9		22.9	20.3		21.0
Solutions renewed with	(0.0)	(0.55)	No renewal	(0.0)	(0.55)	No renewal
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
December 20	29.8	30.7	28.8	20.7	22.4	21.3
26	33.2	35.1	31.7	21.9	24.3	21.3
1928						
January 3	38.8	42.8	35.6	21.6	28.6	21.3
10	41.8	50.0	39.8	21.8	31.8	21.2
Number of plants in group	5	5	10	5	5	10
Green weight	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Tops	63	66	52	34	49	22
Roots	13	15	18	4	13	5
Total	76	81	70	38	62	27
Dry weight						
Tops	6.9	7.6	5.8	3.6	5.0	3.1
Roots	1.3	1.8	1.5	0.3	1.0	0.3
Total	8.2	9.4	7.3	3.9	6.0	3.4

The upper part of table XII shows the average height per plant in each group on the dates indicated in the first column. For the first six weeks

little variation can be seen between the plants of the first two groups each receiving 0.55 ppm. boron. Likewise little difference exists in the height of the plants of the two groups receiving no boron for this same time period. The plants of the latter two groups are slightly shorter on December 13 than those of the first two groups, but no significant differences can be detected on December 6, one week earlier. On December 13, the first and third groups were divided, thus making six groups. The treatments are indicated in the line between the dates December 13 and 20 of the table. As shown in the observation of January 10, four weeks after these changes were made, the effect of boron on stem elongation is very interesting. The average height of plants of group one, receiving no boron, is approixmately 8 cm. less than their controls. The original second group in which no solutions were renewed but which originally contained 0.55 ppm. boron has practically the same stem height value as the first half of group one. It thus appears as though a deficiency of boron were the limiting growth factor rather than a deficiency of any other element originally contained in the solution since all the elements of solutions in group one were renewed on

FIG. 8. Photograph showing tomato plants grown for six weeks in given concentrations of boron, then changed to other concentrations for a period of four weeks. The following treatments were used (reading from left to right): No boron and no renewal of solution; no boron, but solution renewed; no boron, then solution renewed plus 0.55 ppm. boron.

December 13 and only distilled water added to cultures of the original second group. The green and dry weight data shown in the lower half of the table bring out this point but to a less extent. Turning now to the second part of the table, the groups originally receiving no boron, it will be noted that after six weeks of growth without boron the plants receiving boron for the last four weeks started to grow. By January 10, four weeks later, the plants in the second half of group four were 10 cm. taller than their controls. Both green and dry weight also indicate a remarkable

FIG. 9. Photograph showing new leaf growth in tomato plants after they had been defoliated. All solutions were similar with the exception of their boron content. Reading from left to right: No boron; 0.011 ppm.; 0.55 ppm.

increase in growth due to the action of the boron added to the nutrient solution.

This renewal of growth when the plants have not been deprived of boron for too long a period is shown in figure 8. The plants here shown are representative ones taken from groups receiving the last three treatments indicated in table XII. This remarkable influence exerted by boron on growth, especially on the formation of new cells in the growing points was observed in connection with the experiment from which data on the pectic materials

were obtained. After the leaves had been removed for analysis the defoliated plants were left standing in their respective culture media for four weeks. After this period it was observed that no new shoots had appeared on the plants of the boron-deficient solutions while new shoots and leaves were abundant on the plants in the group treated with 0.55 ppm. boron. The intermediate group of plants, those receiving 0.011 ppm. boron, contained a few new shoots and leaves. Figure 9 illustrates the appearance of representative plants from each of these three groups.

Discussion

The evidence presented by plant physiologists in recent years is such that boron must be regarded an essential element for the growth and normal development of many of the higher plants. As yet the exact function of this element is not known. To speak of it as stimulating in small concentrations and as toxic in larger concentrations means little as to its fundamental physiological relation to the plant. Toxicity is a matter of relative concentrations in the presence or absence of other substances. Even the "old" nutrient elements are toxic under certain conditions. The authors are in agreement with SOMMER AND LIPMAN (**14**) that to speak of the catalytic effect of an element merely expresses in other language ignorance in respect to its actual function.

BRENCHLEY (**2**) states, "The old 'nutrients' had certain definite characters in common, in that they were essential to plant growth, the growth being in a great degree proportional to the supply, a relatively large amount of the nutrients being not only tolerated but necessary. . . . Even those that cause increased growth or that may be essential for nutrition (boron) are not required in such quantities as potassium, phosphorus, nitrogen, etc., while there is no evidence that growth is proportional to supply." It is true that boron cannot be used in quantities nearly as great as potassium, phosphorus or nitrogen, yet in these experiments with the tomato there is evidence of a quantitative relationship between growth and the amount of boron supplied. Reference to table XI and to figures 5, 6 and 7 clearly show a difference in growth where 0.011 and 0.55 ppm. boron were used.

SOMMER (**13**) found in her work with monocotyledonous plants such as corn, abnormal tillering as well as withering of the growing points of the tops. This interesting growth formation is no doubt related in some way to the peculiar growth forms (see figure 4) produced in the tomato after the growing points of the stems died. Apical dominance enters the problem at this point. As soon as the influence of the terminal shoot on the development of lateral buds is removed, these lateral buds begin their growth. Such dominating influence can be eliminated by removing the terminal

growing point; it amounted to the same thing in the tomato when the terminal shoot died from a cause attributed to boron deficiency. On *a priori* reasoning the abnormal tillering of corn in SOMMER's experiments may in part be explained as a pruning effect; at least there is an interesting similarity in the behavior of these two widely different plants.

Although extremely small quantities of boron are essential to the tomato plant this element must be supplied constantly. There is apparently no reserve built up in the plant for future growth since plants grown for six weeks in solutions containing boron and then grown in boron-deficient solutions soon showed characteristic symptoms of boron-deficient injuries. This is apparent from the data given in table XII and is in agreement with WARINGTON's (**17**) statement, "The fact that boron can be detected in the stem, leaves, and pods of the broad bean implies that the element becomes distributed throughout the plant after absorption; and further, the need for the supply of boron to be maintained during the life of the plant indicates that the initial reserve of the element in the seed is insufficient for the needs of the plant, and that it is in some way fixed and not in a state of circulation."

A vital relationship exists between boron and the conducting tissues of the tomato. If boron is essential for cell division as seems to be the case in the meristematic tissue of growing points, its presence is just as much needed in the cambium cells where phloem and xylem tissues are forming. These are the tissues which together with the tip of the stem show greatest injury from a deficiency of boron. Both the chemical analyses and macroscopic observations indicated a failure on the part of the boron-deficient plants to remove sugar from their leaves. This is apparently related to the broken down condition of the conducting tissues. Microscopic examinations of the petioles and stems of boron-deficient plants showed phloem necrosis. These general observations are in agreement with the anatomical studies of WARINGTON (**17**) on *Vicia faba* grown in boron-deficient solutions.

At first it was thought the characteristic brittleness of stems and petioles of boron-deficient plants was in some way associated with pectic materials. The experimental data, however, fail to substantiate such a theory. The observation by BRENCHLEY (**2**) that boron occurs most abundantly in bark and lignified parts, suggests that brittleness may be related to a lack of proper lignification. No conclusions upon this point appear possible from the lignin-suberin values which we have obtained.

Conclusions

The conclusions drawn from a series of water culture experiments carried out at the Universities of California and Maryland with two varieties

of tomato, Santa Clara Canner and Marglobe, may be summarized as follows:

1. The element boron in a concentration of approximately 0.5 ppm. was found necessary for the normal growth and development of the tomato plants studied.

2. Tomato plants grown in boron-deficient solutions show four distinct types of injury; (a) death of the terminal growing point of stem; (b) breaking down of the conducting tissues in the stem; (c) a characteristic brittleness of stem and petiole; and (d) roots of extremely poor growth and of a brownish unhealthy color.

3. As a result of broken down conducting tissues the boron deficient plants differed markedly from normal plants in their chemical composition. Total sugars and starch were more abundant in the leaves and stems of the boron-deficient plants while a greater amount of benzene-soluble matter was found in the leaves of the normal plants and in the stems of the boron-deficient plants.

4. Evidence is presented which shows the possibility of a quantitative relationship existing between the amount of growth and the amount of boron present in the nutrient media.

5. A concentration of 5.5 ppm. boron in the nutrient solution was toxic to the tomato plants. Symptoms of boron toxicity are quite different from deficiency injuries.

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THE UNIVERSITY OF MARYLAND,
THE UNIVERSITY OF CALIFORNIA.

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RELATION BETWEEN TOP AND ROOT SIZE IN HERBACEOUS PLANTS*

JOHN W. CRIST AND G. J. STOUT

In his Treatise on the Physiology of Plants SORAUER affirms that the plant must not be looked upon as an unchangeable organism, restricted to a definite form, but as being plastic, capable of further modification in any of its parts; its usual shape being susceptible to alteration, within certain limits, as if it were made of wax. This principle has been substantiated by the practical gardener, who, besides seeking to grow herbaceous plants in situations to which they are the best adapted by nature, often endeavors to cultivate them successfully in special localities, and, in all localities, seeks to render them more useful to his purposes.

Perhaps chief among the attempts of the gardener at altering normal physiological functions and interfering with the ordinary course of development is that of remodelling certain plants with respect to the relative size of top and root. His success in this effort has been remarkable; so much so that there might seem to be an attainable "ideal balance" between size of top and root for every particular desire or necessity. However, the limits mentioned by SORAUER must exist, making it impossible to proceed beyond a certain point with a given species, and also rendering it likely that even within the limits set by nature, increasing or decreasing the normal development of either top or root in order to favor the other part may result in danger to the plant as a whole except this be attempted by persons who thoroughly understand the plant and its conditions of life and vigor. Obviously, the aerial and subterranean portions of a higher land plant are mutually dependent, since the roots furnish the raw nutrients and water obtained from the soil and often serve as places of storage, while the tops supply the elaborated organic compounds essential for growth. It is of practical significance and also of considerable scientific interest to determine the nature of this relationship, or, more specifically, to determine how much variation in this state of reciprocity occurs naturally, and how much may be induced experimentally.

Literature

Interest in the proportionate size of the top and root of the plant is of long standing. The following quotation is from the ancient writings of VARRO (7): "In autumn and winter the roots develop more than does the leaf of the plant because they are nourished by the warmth of the roof of

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earth, while the leaf above is cut down by the frosty air. We can learn this by observation of the wild vegetation which grows without the intervention of man, for the roots grow more rapidly than that which springs from them, but only so far as they are actuated by the rays of the sun. There are two causes of the growth of roots, the vitality of the root itself by which nature drives it forward and the quality of the soil which yields a passage more easily in some conditions than in others."

These early observations have been followed in more recent times by numerous experimental studies on the relation of top and root size, use having been made of many different species and varieties of herbaceous plants. A chronological review of the literature shows by way of generalization that there has been an increasing tendency to seek the causes underlying variations in relative growth of top and root, and also to subject the data obtained to more complete mathematical analysis and interpretation. These trends at their highest present points are exemplified in reports such as those made by WEAVER (**9**), WEAVER and his associates (**8**), TURNER (**6**) and PEARSALL (**3, 4**), the latter having concluded that the relative sizes of plant organs conform to the equation $X = CY^k$, where X and Y are the sizes of the organs, C is a constant expressing their relative sizes, and indicates the ratio of their relative logarithmic growth rates and the spatial arrangements of their meristems. Obviously, this formula sets forth the relationship that the logarithm of stem weight is directly proportional to the logarithm of root weight. When the two time-series of logarithms are plotted against each other the result is a straight line.

In general, the available experimental evidence leads to the conclusion that herbaceous plants exhibit specific and varietal differences with respect to relative development of top and root under uniform environal conditions. However, few if any of the many types are non-plastic, that is, do not react to environmental changes by way of showing variations in the relative sizes of aerial and underground parts. The more intensive studies, particularly those made on growth rates, indicate that though variation occurs there is a persistent tendency towards positive correlation. The present writers have not examined growth rates but have obtained final weights on the tops and roots of lettuce, radish and tomato plants from a number of different experiments and have treated the data as shown later.

Methods of procedure

EXPERIMENTAL

In some of the experiments to be reported the plants were grown in greenhouse flats; in others in flower pots of different sizes. Whether grown in flats or pots the containers were set on boards to prevent the exit of the

roots. Where pots were used, these were set on the bottom of the greenhouse bench and buried to their rims in moist sand. Any objection to the confinement of the roots in such containers can be met by the statement that this was simply one of the conditions under which top-root relations were measured. If the ultimate sizes of top and root are definitely and inevitably correlated, then the type of container might alter the degree of correlation but not destroy it nor change its character qualitatively. Furthermore, the use of flats and pots made it possible to recover the root systems readily and *in toto*. All the experiments were conducted in the greenhouse and every precaution taken to maintain uniform conditions of environment while any one of them was under way.

When the plants were grown in pots, the roots were obtained by freeing the whole mass from the pot, immersing it in a large volume of water, and gently kneading it with the hands until practically all of the soil was washed out. Following this, the root system was washed twice more in fresh water and then placed between layers of newspapers to dry off for a given length of time before the weight was taken.

When flats were used as containers, six plants were grown in each flat. The process for securing the separate root systems was that of dividing the mass of soil into six equal blocks; removing each one of the blocks by placing a square of galvanized iron under it, and immersing the block in water. From this point onward the method was the same as described previously for the plants grown in pots.

Mathematical

The top-root ratios were calculated in three ways: (1) as the quotient of mean (arithmetic) top to mean (arithmetic) root; (2) as the arithmetic mean of the individual top-root ratios, and (3) as the geometric mean of the individual ratios. The results from the use of these three methods of calculation on the same data differ sufficiently to necessitate making a choice among them. The properties of the geometric mean, when dealing with ratios, are such as to commend its use in preference to the others. On this mean and its probable error see Yule (**10**), Kelley (**2**), and Crist (**1**).

When the number of individuals in any lot of plants was less than 25, Pearson's (**5**) factors for the correction of the standard deviation were used and the corrected standard deviation substituted in all other calculations where the employment of the standard deviation was required.

The product-moment formula ($r = \frac{p}{\sigma_x \cdot \sigma_y}$) was used in calculating the coefficients of correlation. The probable error of r was obtained with the formula: $P.E._r = \frac{1 - r^2}{\sqrt{N}} \cdot 0.6745$.

No difference less than three times its probable error was considered significant, the probable error of the difference having been determined through the formula: $P.E._d = 0.6745\sqrt{\sigma_1^2 + \sigma_2^2}$.

Interpretation of the significance of the coefficient of correlation was made according to the following rules: (a) If r is 0.5 (plus or minus) or greater, correlation is practically certain. (b) In order to be reliable r must be at least four times its probable error. (c) If, under various conditions of experimentation, r persists in having the same sign, though not always significant in itself, there is some evidence of a general though weak relationship of the character of this sign between the two variables.

Experiments with lettuce (Grand Rapids Forcing variety)

Use of raw sulphur and superphosphate (c.p. and commercial.)—The seed was sown broadcast in greenhouse flats, the seedlings selected at the proper time, and transplanted to other flats (6 per flat) in which they were grown for a period of 51 days. The soil was a fertile fine sandy loam. The data appear in table I.

In each case shown in table I the ratio of the average top to average root R_1 is less than R (the arithmetic mean of the ratios for the plants individually), while Mg lies between these two. The probable error for any Mg is higher than for the corresponding R. This is the relationship between the three forms of average which holds as a general rule. Accordingly, only the geometric mean will be given and used hereafter in making comparisons.

The number of plants per lot was small, by design. This necessitated large differences, if they were to be significant. Under these conditions, superphosphate when applied either alone or along with raw sulphur, except in lot 4 where 800 pounds of the sulphur per acre were used, significantly increased the top-root ratio as compared with lots 1 and 2 where sulphur only was applied.

The ratios do not vary directly with the total weights of average plants. The smallest plants have the highest ratio, the largest plants have the second largest ratio, while the plants intermediate in size have the lowest ratios.

With so few plants none of the coefficients of correlation, including that for all the plants taken together, is significant. However, it is noteworthy that all of them are positive in character, one (lot 5) being above 0.5, and also that they do not vary consistently in magnitude with either the top-root ratios or with the average total weights of the plants.

Influence of calcium nitrate, potassium chloride and superphosphate.—The plants were grown in a very poor medium sandy loam soil in six inch pots. The nutrient treatments were applied in solution form at the beginning of the experiment. Further details and other data for this

experiment may be found in Michigan Experiment Station Tech. Bull. 74. 1926. See table II for the results.

TABLE I

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	SOIL TREATMENT	AVERAGE GREEN WEIGHT		R_1[a]	R[a]	Mg[a]	r[a]
			TOP	ROOT				
		lbs. per acre	*gm.*	*gm.*				
1	6	Sulphur, 400	86.8 ± 3.63	11.5 ± 0.31	7.52	7.56 ± 0.265	7.48 ± 0.270	0.433 ± 0.224
2	6	Sulphur, 800	89.3 ± 1.07	12.2 ± 1.07	7.33	7.54 ± 0.389	7.44 ± 0.401	0.169 ± 0.267
3	6	c.p. $CaH_4(PO_4)_2$, 1200	93.5 ± 3.43	10.0 ± 0.06	9.35	9.57 ± 0.470	9.46 ± 0.479	0.367 ± 0.238
4	6	c.p. $CaH_4(PO_4)_2$, 1200 plus sulphur, 800	90.6 ± 4.00	10.8 ± 0.02	8.39	8.38 ± 0.330	8.32 ± 0.337	0.333 ± 0.245
5	6	Commercial $CaH_4(PO_4)_2$, 1200	99.2 ± 2.52	9.3 ± 0.06	10.60	10.94 ± 0.550	10.80 ± 0.565	0.578 ± 0.186
6	6	Commercial $CaH_4(PO_4)_2$, 1200 plus sulphur, 400	84.0 ± 2.51	7.3 ± 0.07	11.50	12.53 ± 1.170	12.02 ± 1.255	0.296 ± 0.251
All	36							0.319 ± 0.100

[a] In all tables "R_1" is ratio of average top to average root; "R" average ratio of individual plants; "Mg" geometric mean of individual ratios; "r" coefficient of correlation.

TABLE II

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
			TOP	ROOT	WHOLE PLANT		
		lbs. per acre	*gm.*	*gm.*	*gm.*		
1	14	Check	7.4 ± 0.27	7.9 ± 0.22	15.3	0.93 ± 0.029	0.481 ± 0.139
2	7	$Ca(NO_3)_2$, 100	14.6 ± 0.83	11.7 ± 0.74	26.3	1.25 ± 0.023	0.761 ± 0.107
3	7	$Ca(NO_3)_2$, 300	14.5 ± 0.84	9.1 ± 0.39	23.6	1.58 ± 0.102	0.276 ± 0.235
4	7	$Ca(NO_3)_2$, 600	20.4 ± 1.19	12.3 ± 0.53	32.7	1.64 ± 0.103	0.398 ± 0.214
5	7	$Ca(NO_3)_2$, 600 plus KCl, 300	22.9 ± 1.29	13.2 ± 0.25	36.1	1.71 ± 0.077	0.591 ± 0.166
6	7	$Ca(NO_3)_2$, 600 plus $CaH_4(PO_4)_2$, 300	35.0 ± 1.75	18.1 ± 1.06	53.1	1.95 ± 0.077	0.621 ± 0.156
7	7	$Ca(NO_3)_2$, 600 plus KCl, 300 plus $CaH_4(PO_4)_2$, 300	33.9 ± 2.16	16.6 ± 0.91	50.5	2.03 ± 0.166	0.243 ± 0.239
All	56						0.879 ± 0.020

Again, as in the preceding experiment, the number of plants per lot, except lot 1, was small. Nevertheless, table II shows that any nutrient treatment increased the top-root ratio and the ratios show an unmistakable tendency to be continuously higher as more complete fertilization of the soil obtains. The average weight of plant increases along with the ratios except in lots 3 and 7. In these cases the increased ratio does not indicate an absolute weight of top greater than in the preceding lot but is brought about by a reduction in the root which exceeds that in the top, thus leaving the top actually smaller, but still relatively larger than the root.

Though but one of the coefficients of correlation (lot 2) is significant, all are positive in sign; three of them (lots 2, 5, 6) being greater than 0.5. Here, as in the former experiment, these coefficients sustain no uniform relation to either the top-root ratios or the average total weights of the plants. When thrown together into one group, the 56 plants show strong positive correlation, the coefficient being 0.879 ± 0.020.

USE OF ACID AND NEUTRAL SOIL WITH APPLICATIONS OF HYDRATED LIME AND SUPERPHOSPHATE.—Complete details of this experiment, which consisted primarily in a study of the acid tolerance of lettuce, can be found in Michigan State College Experiment Station Tech. Bull. 71. 1926, pages 14–18. The plants were grown in flats. Two types of soil were used. One soil was a mixture of two-thirds muck and one-third sand with a lime requirement of 20,000 pounds $Ca(OH)_2$ (JONES method). The second soil was a neutral fine sandy loam. The data, presented in table III, were taken when the plants had reached vegetative maturity. The number of plants per lot was larger than in either of the two former experiments.

The twenty-seven lots of plants listed in table III fall into five groups, namely, lots 1 to 6 with increasing amounts of lime; lots 7 to 10 with increasing applications of both lime and superphosphate; lots 12 to 17 with superphosphate constant while the lime treatments increase in size; lots 19 to 22 on neutral loam soil with superphosphate constant and lime applications increasing in size; lots 23 to 27 on neutral loam soil with increasing quantities of lime. Within any one of the five groups, the top-root ratios are approximately equal for the several lots, while at the same time, the average size of whole plants decreases with increased size of application of the materials used for soil treatment, due to both top and root being progressively smaller to about the same degree.

The effect of soil type is very pronounced. The top-root ratios on the acid muck-and-sand mixture are conspicuously higher than on the neutral sandy loam. This holds true where comparable treatments were used as well as for the checks. Relative root development in the acid muck-and-sand soil mixture was greatly reduced. This went to a very great extreme

TABLE III

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	SOIL TREATMENT[c]	AVERAGE GREEN WEIGHT			Mg	r
			TOP	ROOT	WHOLE PLANT		
		tons per acre	*gm.*	*gm.*	*gm.*		
1[a]	24	Check	65.7 ± 2.24	8.8 ± 0.34	74.5	7.58 ± 0.254	0.540 ± 0.100
2	12	$Ca(OH)_2$, 2.5	70.3 ± 2.65	10.2 ± 0.47	80.5	6.94 ± 0.365	0.314 ± 0.176
3	12	$Ca(OH)_2$, 5.0	58.7 ± 1.93	9.7 ± 0.36	68.4	6.08 ± 0.368	−0.265 ± 0.181
4	12	$Ca(OH)_2$, 7.5	38.2 ± 1.45	6.0 ± 0.24	44.2	6.40 ± 0.237	0.408 ± 0.163
5	12	$Ca(OH)_2$, 10.0	26.0 ± 1.24	3.2 ± 0.19	29.2	8.32 ± 0.555	0.443 ± 0.156
6	12	$Ca(OH)_2$, 12.5	15.4 ± 0.94	1.8 ± 0.16	17.2	9.10 ± 0.742	0.678 ± 0.105
7	12	$Ca(OH)_2$, 3.4 plus $CaH_4(PO_4)_2$, 2	69.4 ± 3.03	5.4 ± 0.22	72.7	12.96 ± 1.821	0.358 ± 0.170
8	12	$Ca(OH)_2$, 6.8 plus $CaH_4(PO_4)_2$, 4	70.7 ± 2.03	6.4 ± 0.32	77.1	11.36 ± 0.567	0.447 ± 0.156
9	12	$Ca(OH)_2$, 10.0 plus $CaH_4(PO_4)_2$, 6	60.8 ± 1.94	4.9 ± 0.34	65.7	12.89 ± 1.008	0.287 ± 0.179
10	12	$Ca(OH)_2$, 13.4 plus $CaH_4(PO_4)_2$, 8	56.0 ± 2.11	4.3 ± 0.29	60.3	13.41 ± 0.613	0.618 ± 0.121
11	18	c.p. KH_2PO_4, 8	21.2 ± 1.71	3.2 ± 0.19	24.4	6.27 ± 0.414	0.664 ± 0.089
12	12	$CaH_4(PO_4)_2$, 8	24.7 ± 1.42	4.2 ± 0.28	28.9	5.95 ± 0.350	0.608 ± 0.123
13	12	$Ca(OH)_2$, 2.5 plus $CaH_4(PO_4)_2$, 8	81.8 ± 2.08	11.3 ± 0.46	93.1	7.33 ± 0.193	0.712 ± 0.096
14	12	$Ca(OH)_2$, 5.0 plus $CaH_4(PO_4)_2$, 8	75.3 ± 2.04	7.7 ± 0.31	83.0	9.94 ± 0.666	−0.460 ± 0.154
15	12	$Ca(OH)_2$, 7.5 plus $CaH_4(PO_4)_2$, 8	65.9 ± 1.30	10.6 ± 0.59	76.5	6.39 ± 0.309	0.522 ± 0.142
16	12	$Ca(OH)_2$, 10.0 plus $CaH_4(PO_4)_2$, 8	60.3 ± 2.23	8.8 ± 0.41	69.1	6.89 ± 0.361	0.356 ± 0.170
17	12	$Ca(OH)_2$, 12.5 plus $CaH_4(PO_4)_2$, 8	72.1 ± 2.58	11.1 ± 0.45	83.2	6.52 ± 0.411	−0.075 ± 0.194
18[b]	12	$CaH_4(PO_4)_2$, 2	16.9 ± 0.85	7.3 ± 0.36	24.2	2.30 ± 0.082	0.645 ± 0.114
19	12	$Ca(OH)_2$, 1 plus $CaH_4(PO_4)_2$, 2	18.1 ± 0.84	7.8 ± 0.27	25.9	2.28 ± 0.199	0.729 ± 0.091
20	12	$Ca(OH)_2$, 2 plus $CaH_4(PO_4)_2$, 2	21.5 ± 1.36	6.7 ± 0.25	28.2	3.15 ± 0.162	0.622 ± 0.119
21	12	$Ca(OH)_2$, 3 plus $CaH_4(PO_4)_2$, 2	17.9 ± 0.94	6.7 ± 0.36	24.6	2.65 ± 0.125	0.407 ± 0.163
22	12	$Ca(OH)_2$, 4 plus $CaH_4(PO_4)_2$, 2	14.0 ± 0.82	4.7 ± 0.35	18.7	3.12 ± 0.356	0.508 ± 0.144
23	12	Check	17.9 ± 0.83	8.0 ± 0.40	25.9	2.24 ± 0.090	0.605 ± 0.123
24	12	$Ca(OH)_2$, 1	18.7 ± 1.15	7.5 ± 0.28	26.2	2.42 ± 0.120	0.589 ± 0.127
25	12	$Ca(OH)_2$, 2	14.3 ± 0.93	5.6 ± 0.44	19.9	2.62 ± 0.121	0.748 ± 0.086
26	12	$Ca(OH)_2$, 3	9.6 ± 0.68	3.1 ± 0.11	12.7	2.99 ± 0.196	0.439 ± 0.157
27	12	$Ca(OH)_2$, 4	7.6 ± 0.48	2.6 ± 0.17	10.2	3.02 ± 0.098	0.797 ± 0.071
All	342						0.543 ± 0.026

[a] Lots 1 to 17 inclusive, soil was muck-and-sand mixture.

[b] Lots 18 to 27 inclusive, soil was neutral sandy loam.

[c] All nutrient substances of commercial grade, unless noted otherwise.

in lots 7 to 10 where the lime and the superphosphate were increased together.

In regard to the twenty-seven coefficients of correlation three, none of which is at all significant, are negative. Twenty-four of the coefficients, ten of which are above 0.5 and significant, are positive. Variation in the size of the coefficients is not consistent to any important extent with the soil treatments or the changes in the size of the plants and the ratios produced by these treatments. Correlation with all the plants taken in a single group is positive and clearly significant (0.543 ± 0.026).

INFLUENCE OF LENGTH OF DAY AS MODIFIED BY NUTRIENT TREATMENT.—The plants were grown singly, to vegetative maturity, in six-inch pots, the soil being a fine sand, low in fertility. Various nutrients were applied in different ways. The extra illumination of the plants designated as long-day plants was accomplished by means of 1000 watt Mazda lamps, socketed in porcelain enameled steel dome reflectors suspended at a height of 4.5 feet above the bench. Three of these bulbs, evenly spaced, were used over a bench 21 feet long and 5 feet wide, the pots containing the plants being distributed so as to give even illumination and also being systematically shifted about in their places once each week. The experiment was conducted during the short cloudy days of the winter season. The day for the long-day plants was lengthened 6 hours as an average. The plants of the short-day lots were covered each day at 3:00 P. M. and left covered until 9 o'clock the following morning. The covers consisted of specially built light-tight boxes each 10 feet long, 5 feet wide and 3 feet high, with light-tight ventilators arranged in each end. See table IV for the results.

An examination of table IV makes it clear that the top-root ratios were increased as the period of illumination was shortened. The differences for lots 1, 6 and 7, lots 2 and 8, and lots 3 and 9 are large and certainly significant. In the long-day group the nutrient treatments elevated the ratios decidedly. The increases in the short-day group are only apparent. Within groups, the increased ratios are associated with increased size of plant and greater actual weight of top, though the three largest ratios (lots 7, 8, 9) obtain with plants among the smallest in size. While the nitrate treatments of the short-day plants elevated the top-root ratios apparently, large plants were not produced as with the long-day treatments. Though raw materials from the soil have been sufficient, assimilable food products were not ample to give greater total growth more evenly distributed between top and root.

All of the coefficients of correlation are positive, three of them (lots 1, 6 and 9) giving evidence of certainty. The coefficient (0.685 ± 0.026) for all the plants, thrown together, is such as to make positive correlation certain.

TABLE IV

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	DAY	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
				TOP	ROOT	WHOLE PLANT		
			gm. per pot	*gm.*	*gm.*	*gm.*		
1	21	Long	Check	11.2 ± 0.32	14.5 ± 0.65	25.7	0.79 ± 0.023	0.700 ± 0.075
2	21	"	$Ca(NO_3)_2$, 1	49.8 ± 1.58	18.9 ± 0.84	68.7	2.71 ± 0.177	0.355 ± 0.129
3	21	"	[a]$Ca(NO_3)_2$, 1	54.2 ± 2.28	11.3 ± 0.62	65.5	4.90 ± 0.373	0.363 ± 0.128
4	21	"	$Ca(NO_3)_2$, 1 plus $CaH_4(PO_4)_2$, 1	58.8 ± 1.58	15.1 ± 0.47	73.9	3.94 ± 0.171	0.133 ± 0.144
5	21	"	[a]$Ca(NO_3)_2$, 1 plus [a]$CaH_4(PO_4)_2$, 1	75.2 ± 1.77	16.7 ± 0.84	91.9	4.72 ± 0.320	0.385 ± 0.125
6	15	Normal	Check	11.0 ± 0.62	4.5 ± 0.47	15.5	2.84 ± 0.590	0.713 ± 0.086
7	20	Short	Check	8.5 ± 0.32	1.4 ± 0.13	9.9	6.88 ± 1.101	0.417 ± 0.125
8	22	"	$Ca(NO_3)_2$, 1	15.1 ± 0.52	1.5 ± 0.11	16.6	10.84 ± 1.000	0.352 ± 0.126
9	22	"	[a]$Ca(NO_3)_2$, 1	13.4 ± 0.59	1.7 ± 0.14	15.1	8.74 ± 0.701	0.582 ± 0.095
All	184							0.685 ± 0.026

[a] Applied cumulatively in fourth portions.

INFLUENCE OF LENGTH OF DAY IN CONJUNCTION WITH NUTRIENT TREATMENTS (SECOND EXPERIMENT).—This experiment differed from the preceding one in that it was performed during the spring season (April 17 to May 23), the former having been conducted in midwinter. The short-day plants had a day of eight hours, the long-day plants 16 to 18 hours, while the normal day was approximately 10 to 12 hours in length. Otherwise, the two experiments were duplicated as to methods. The results are shown in table V.

The data in table V confirm the main conclusion that was evident from the results of the preceding experiment, namely, that the top-root ratio was raised as the day was shortened, either with or without the nitrate treatments. In two of the three groups the nitrate of itself significantly increased the ratio. Again, the highest ratios are for the short-day plants and are associated with the smallest plants which, at the same time, have the lowest actual weight of top, as well as root.

All of the coefficients of correlation are positive, three of them being about 0.5 and no one of these three less than five times its probable error. The coefficient for the 178 plants is positive, more than 0.5, and is 21 times its probable error.

EXPERIMENTS WITH RADISH PLANTS (SCARLET GLOBE VARIETY)

USE OF NUTRIENT SUBSTANCES ON TWO SOILS OF DIFFERENT COMPOSITION.—One of the two soils, the one designated as A in table VI was composed of five parts fine sandy loam and one part medium coarse sand; the other, B, of these types of soil, with the proportions reversed. These soils were treated as shown in table VI, and used in six-inch pots in which the plants were grown singly.

Table VI shows that the nutrient treatments increased the average size of plant on each one of the two soils but the top-root ratio was not changed significantly. With one exception (lot 6) the average plant was larger on soil B than soil A, while at the same time the ratio of top to root is consistently lower, though the differences are not strictly real except as between lots 1 and 2.

The ten coefficients of correlation are positive; seven of them being above 0.5. Of these seven, three are fully significant. The coefficient (0.694 ± 0.031) for all the plants indicates certain positive correlation.

EFFECT OF LENGTH OF DAY AS MODIFIED BY NUTRIENT TREATMENTS

This experiment was made simultaneously with the one on lettuce, the data for which are contained in table IV, using the same general methods of procedure. The period of illumination for the long-day plants was six

TABLE V

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	DAY	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
				TOP	ROOT	WHOLE PLANT		
			gm. per pot	*gm.*	*gm.*	*gm.*		
1	30	Long	Check	54.5 ± 1.43	34.3 ± 0.74	88.8	1.58 ± 0.033	0.625 ± 0.076
2	30	"	$NaNO_3$, 1	79.4 ± 0.85	39.4 ± 0.72	118.8	2.03 ± 0.031	0.543 ± 0.086
3	29	Normal	Check	55.4 ± 1.21	26.3 ± 0.62	81.7	2.11 ± 0.055	0.324 ± 0.111
4	30	"	$NaNO_3$, 1	83.0 ± 1.13	39.8 ± 0.99	122.8	2.12 ± 0.053	0.340 ± 0.109
5	29	Short	Check	46.9 ± 0.88	17.8 ± 0.51	64.7	2.67 ± 0.064	0.491 ± 0.095
6	30	"	$NaNO_3$, 1	68.2 ± 1.19	17.3 ± 0.46	85.5	3.98 ± 0.108	0.295 ± 0.113
All	178							0.573 ± 0.027

TABLE VI

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR RADISH PLANTS

LOT	NUMBER OF PLANTS	SOIL	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
				TOP	ROOT	WHOLE PLANT		
			gm. per pot	*gm.*	*gm.*	*gm.*		
1	12	A	Check	3.3 ± 0.20	6.2 ± 0.19	9.5	0.51 ± 0.026	0.763 ± 0.082
2	15	B	Check	3.2 ± 0.16	7.8 ± 0.42	11.0	0.41 ± 0.017	0.584 ± 0.115
3	12	A	$Ca(NO_3)_2$, 1	3.5 ± 0.19	7.0 ± 0.43	10.5	0.49 ± 0.057	0.356 ± 0.170
4	15	B	$Ca(NO_3)_2$, 1	5.6 ± 0.33	14.3 ± 0.78	19.9	0.39 ± 0.020	0.621 ± 0.107
5	12	A	KCl, 1	3.6 ± 0.26	7.7 ± 0.38	11.3	0.45 ± 0.034	0.590 ± 0.127
6	15	B	KCl, 1	2.9 ± 0.13	8.0 ± 0.38	10.9	0.36 ± 0.020	0.566 ± 0.119
7	12	A	$Ca(NO_3)_2$, 1 plus KCl, 1	3.9 ± 0.34	7.4 ± 0.28	11.3	0.50 ± 0.046	0.265 ± 0.181
8	15	B	$Ca(NO_3)_2$, 1 plus KCl, 1	3.6 ± 0.17	9.5 ± 0.36	13.1	0.38 ± 0.019	0.533 ± 0.125
9	6	A	$CaH_4(PO_4)_2$, 1	4.2 ± 0.24	8.0 ± 0.66	12.2	0.53 ± 0.029	0.586 ± 0.181
10	15	B	$CaH_4(PO_4)_2$, 1	4.2 ± 0.25	9.1 ± 0.54	13.3	0.47 ± 0.061	0.387 ± 0.148
All	129							0.694 ± 0.031

TABLE VII

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR RADISH PLANTS

LOT	NUMBER OF PLANTS	DAY	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
				TOP	ROOT	WHOLE PLANT		
			gm. per pot	*gm.*	*gm.*	*gm.*		
1	22	Long	Check	3.5 ± 0.09	7.0 ± 0.26	10.5	0.51 ± 0.023	0.261 ± 0.134
2	21	"	$Ca(NO_3)_2$, 1	7.6 ± 0.46	11.8 ± 0.51	19.4	0.62 ± 0.031	0.637 ± 0.088
3	22	"	KCl, 1	3.5 ± 0.13	6.1 ± 0.39	9.6	0.61 ± 0.044	0.487 ± 0.110
4	22	"	$Ca(NO_3)_2$, 1 plus KCl, 1	8.6 ± 0.43	12.3 ± 0.53	20.9	0.70 ± 0.059	0.212 ± 0.138
5	15	Normal	Check	2.1 ± 0.03	3.2 ± 0.19	5.3	0.67 ± 0.059	0.131 ± 0.171
6	16	Short	Check	1.6 ± 0.09	1.1 ± 0.14	2.7	1.74 ± 0.216	0.511 ± 0.125
7	16	"	$Ca(NO_3)_2$, 1	2.2 ± 0.19	1.1 ± 0.11	3.3	2.10 ± 0.486	0.444 ± 0.136
8	14	"	KCl, 1	1.6 ± 0.11	1.2 ± 0.12	2.8	1.62 ± 0.249	0.558 ± 0.124
All	148							0.824 ± 0.018

hours in excess of the normal day, while that for the short-day plants was four to five hours less than normal. The soil was a medium coarse sand, low in fertility. Table VII gives the results.

With these plants (table VII), as with the lettuce plants (table IV), the top-root ratio increased as the day was shortened, the difference between lot 1 and lot 6 being highly significant. The increases in the ratio are accompanied by decreases in plant size, development in lots 6, 7 and 8 being very meager. Increases in the ratio due to nutrient treatments are only apparent. As to size of plant, the nitrate was effective, more effective with the long than short day.

Each of the eight coefficients of correlation is positive in character. Among the three that exceed 0.5, lot 2 is significant. The coefficient for the plants as a whole (0.824 ± 0.018) is especially high and reliable.

Experiments with tomato plants

Effect of size of container on top-root relation.—Pure line seed of the John Baer variety was sown on July 28. On August 14, the seedlings were potted singly in three-inch pots and remained in these until September 7. On the latter date, 51 of the plants were selected at random and weights of their tops and roots secured. Forty-one plants were shifted to five-inch pots, 41 to eight-inch pots, and 41 to 10-inch pots on September 9. The soil, a fine sandy loam, high in organic matter, was the same for each of the four lots. No manuring of any kind was done. The experiment was concluded on October 10, when the plants in the five-inch pots were just beginning to show symptoms of deterioration because of becoming pot-bound. The leaves and stem were weighed separately. See table VIII for the results.

The data in table VIII make it practically certain that the ratio of top to root rose as larger pots were used. This holds true whether the calculations are based on whole top, on stem, or on leaves. The plants grew to greater size in the larger containers, with both stem and leaves increasing in weight and each of these making greater relative growth than the root. The leaves constitute a greater percentage of the entire top than the stem, and gain in this respect occurs while the stem loses as the pots are larger. Furthermore, the gain in size relative to the roots is greater for the leaves than it is for the stem. The ratio of top to root for lot 2 as compared with lot 1 is not significantly different, while with lots 3 and 4 the ratios are clearly higher.

All of the coefficients of correlation are positive and all fully significant. Six of the nine are above 0.5. Though the differences are not always equal to three times their probable errors, the indication that the degree of corre-

TABLE VIII

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR TOMATO PLANTS

LOT	NUMBER OF PLANTS	SIZE OF POT	AVERAGE GREEN WEIGHT				Mg	r
			AERIAL PART*		ROOT	TOTAL		
		inches		*gm.*	*gm.*	*gm.*		
1	51	3	Top	4.7 ± 0.24	1.8 ± 0.09	6.5	2.19 ± 0.055	0.899 ± 0.018
2	41	5	Top	25.3 ± 1.27	12.5 ± 0.34	37.8	1.94 ± 0.060	0.718 ± 0.051
			Stem	10.6 ± 0.40	12.5 ± 0.34	23.1	0.84 ± 0.016	0.793 ± 0.039
			Leaves	14.7 ± 0.90	12.5 ± 0.34	27.2	1.10 ± 0.049	0.663 ± 0.059
3	41	8	Top	57.0 ± 1.47	19.1 ± 0.35	76.1	2.96 ± 0.074	0.471 ± 0.081
			Stem	22.7 ± 0.57	19.1 ± 0.35	41.8	1.18 ± 0.028	0.504 ± 0.078
			Leaves	34.2 ± 0.94	19.1 ± 0.35	53.4	1.78 ± 0.043	0.462 ± 0.083
4	41	10	Top	82.9 ± 0.77	26.3 ± 0.36	109.2	3.17 ± 0.043	0.457 ± 0.083
			Stem	30.2 ± 0.35	26.3 ± 0.36	56.5	1.15 ± 0.049	0.509 ± 0.078
			Leaves	52.7 ± 0.55	26.3 ± 0.36	79.0	2.01 ± 0.032	0.345 ± 0.092
All	174	All	Top					0.948 ± 0.005
All	123	All	Stem					0.890 ± 0.013
All	123	All	Leaves					0.881 ± 0.013

* Part of plant set in ratio to and correlated with root.

lation lessened as the containers were larger and that the correlation between root and stem was the most pronounced, is quite certain. This means that the degree of correlation lessened as the plants became larger and the ratios of aerial to underground parts increased. Correlation of top and root for the entire number of plants (174) is nearly perfect, while tending to be slightly less for stem and root, and for leaves and root.

INFLUENCE OF DEFLOWERING ON PRUNED AND UNPRUNED PLANTS.—In this experiment, the plants were of the Grand Rapids Forcing variety and were brought along to the stage for transplantation to pots in the same manner as those of the preceding experiment. They were transplanted to six-inch pots and grown therein on a fine sandy loam soil, well supplied with organic matter, for a period of 33 days. One lot of 84 plants was kept trained to a single stem, another lot of the same number allowed to go unpruned. Forty-two plants of each of these two groups had the flowers of the clusters (3 clusters mostly) removed as they opened. See table IX for the data.

Table IX shows some differences which appear consistent, though none of them is great enough to be considered significant. The plants are larger and the ratios higher for the unpruned plants than for the pruned and in both of these two groups larger plants with higher ratios obtain for those from which the flowers were not removed. Thus, higher ratios are apparently associated with the heavier plants.

With the eighteen coefficients of correlation, thirteen are greater than 0.5, and no one of the eighteen is rendered unreliable by the size of its probable error. Though the differences in the coefficients are consistent, being higher for the unpruned than the pruned, and higher for the plants from which the flowers were removed, they are too small, without exception, to be significant.

EFFECT OF BOTTOM HEAT ON PRUNED AND UNPRUNED PLANTS.—The work here differed from that for which the results have been given in table IX only in that bottom heat was supplied to the bench wherein the pots were placed. This elevated the temperature of the plants as a whole but especially that of the soil and the roots it contained. The arrangement for bottom heat was that of steam pipes swung under the bench at a distance of about 18 inches from the bottom of the bench, and heavy burlap, reaching to the floor, hung around the sides of the bench. Heat was kept on during the nights, sufficiently to hold the temperature of the moist sand about the pots at a point averaging about 15 degrees above that of another bench not provided with bottom heat. The results are presented in table X.

In table X, the top-root ratios are higher for the unpruned than the pruned plants, though the differences are small and insignificant. Comparing the data with those from table IX for corresponding groups of plants

TABLE IX

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR TOMATO PLANTS

Lot	Number of plants	Group	Flowers	Average green weight				Mg.	r
				Aerial part*		Root	Total		
					gm.	gm.	gm.		
1	42	Pruned	Off	Top	27.4 ± 0.78	9.2 ± 0.17	36.6	2.92 ± 0.067	0.573 ± 0.070
				Stem	12.8 ± 0.29	9.2 ± 0.17	22.0	1.36 ± 0.025	0.644 ± 0.061
				Leaves	14.7 ± 0.52	9.2 ± 0.17	23.9	1.54 ± 0.047	0.501 ± 0.078
2	42	Pruned	On	Top	31.2 ± 1.02	10.1 ± 0.21	41.3	3.01 ± 0.105	0.510 ± 0.077
				Stem	14.0 ± 0.38	10.1 ± 0.21	24.1	1.37 ± 0.038	0.565 ± 0.070
				Leaves	17.2 ± 0.67	10.1 ± 0.21	27.3	1.63 ± 0.070	0.460 ± 0.082
All	84			Top					0.405 ± 0.061
				Stem					0.444 ± 0.059
				Leaves					0.362 ± 0.064
3	42	Unpruned	Off	Top	33.7 ± 1.09	10.4 ± 0.22	44.1	3.17 ± 0.081	0.668 ± 0.057
				Stem	15.9 ± 0.46	10.4 ± 0.22	26.3	1.51 ± 0.032	0.696 ± 0.054
				Leaves	17.8 ± 0.65	10.4 ± 0.22	28.2	1.64 ± 0.050	0.626 ± 0.060
4	42	Unpruned	On	Top	34.7 ± 1.19	9.8 ± 0.20	44.5	3.44 ± 0.114	0.520 ± 0.076
				Stem	16.3 ± 0.48	9.8 ± 0.20	26.1	1.63 ± 0.046	0.549 ± 0.073
				Leaves	18.5 ± 0.73	9.8 ± 0.20	28.3	1.80 ± 0.077	0.463 ± 0.082
All	84			Top					0.577 ± 0.049
				Stem					0.607 ± 0.047
				Leaves					0.526 ± 0.053

* Part of plant set in ratio to and correlated with root.

TABLE X

TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR TOMATO PLANTS

LOT	NUMBER OF PLANTS	TREATMENT (BOTTOM HEAT)	AVERAGE GREEN WEIGHT				Mg	r
			AERIAL PART*		ROOT	TOTAL		
				gm.	gm.	gm.		
1	47	Unpruned	Top	44.7 ± 0.98	11.8 ± 0.16	56.5	3.70 ± 0.059	0.717 ± 0.069
			Stem	18.2 ± 0.37	11.8 ± 0.16	30.0	1.52 ± 0.021	0.748 ± 0.043
			Leaves	26.5 ± 0.64	11.8 ± 0.16	38.3	2.18 ± 0.042	0.687 ± 0.052
2	51	Pruned	Top	47.7 ± 1.18	13.1 ± 0.20	60.8	3.57 ± 0.067	0.675 ± 0.051
			Stem	19.0 ± 0.39	13.1 ± 0.20	32.1	1.44 ± 0.025	0.614 ± 0.059
			Leaves	28.7 ± 0.82	13.1 ± 0.20	41.8	2.12 ± 0.047	0.675 ± 0.051
All	98	Top						0.690 ± 0.036
		Stem						0.661 ± 0.038
		Leaves						0.671 ± 0.038

* Part of plant set in ratio to and correlated with root.

(lots 2 and 4) it becomes apparent that the use of bottom heat greatly increased the size of average plants. It also led to higher top-root ratios, the difference for the pruned plants being significant.

Each of the nine coefficients of correlation is positive, above 0.5 and fully reliable. None of the apparent differences between the coefficients that are comparable are significant. Better correlation of aerial parts with root obtained than in the preceding experiment, but the differences are only apparent.

Discussion and conclusions

Under any given circumstances an herbaceous plant has a definite possibility as regards total growth and ultimate size. But, while the plant is a unit, functioning and developing as an organic whole, there are distinctions of degree as regards the top and root. Significant variations in the relative distribution of growth between top and root occur naturally and may be readily induced by experimentation. The two parts are dependent, and, in general, they increase together, but not in a continuous and fixed proportion, whatever the time in the life cycle and the environment may happen to be. This is fully substantiated by comparisons of top-root ratios, even when the data are subjected to the standard tests of statistical analysis and the geometric type of average used in the process of interpretation. PEARSALL (**4**) points out that the value of k (ratio of average logarithmic growth rates) in the equation $S = cR^k$ varies with conditions of growth, as well as the type of plant. With etiolated seedlings of *Pisum sativum*, k was 2 to 3 times as great as when the plants were grown in the light. High nitrogen also elevated the value of k.

Though the relative development of top and root varies widely in nature and under experimental conditions, the two parts show a persistent interdependence on the basis of their respective final masses. While this relationship is neither markedly close nor constant in degree, it is present, in general, as a permanent characteristic. Top and root in higher plants, except in a few forms like certain species of *Tillandsia* which are rootless, other organs discharging the functions of roots, vary concomitantly. Generally speaking, correlation between top and root is positive, or in other words, increased size of top is accompanied by increased size of root or *vice versa*. This principle is deducible from the correlation coefficients that have been presented, though not as certainly from the top-root ratios. The ratios are somewhat inadequate, based as they are on the absolute values of weight. The coefficients of correlation which are derived through deviations from the line of average relationship afford the necessary criteria.

An examination of the preceding tables shows little or no tendency of the size of the plant to be dependent upon or governed by the degree of

correlation between root and top with respect to final mass. Correlation is certainly positive in character, but it does not show itself to be stronger as the plants average larger in total weight, nor less as they average smaller. In fact, if there be any relationship at all of this kind, it tends to be negative rather than positive. More often, the higher coefficients of correlation are associated with average plants that are relatively smaller. From this it may be inferred that when conditions are such as to leave the growth of the whole plant less restricted, correlation between top and root appears to be lessened.

These facts help to explain the wide range of plant adaptation observed in nature. In so far as survival, establishment and perpetuation hinge upon changes in relative development of top and root to meet existing conditions, the margin of possibility is quite great. Likewise, the position of the practical grower is rendered advantageous. He has considerable latitude by way of opportunity to alter relative size of top and root in seeking to realize his desires. An "ideal balance" from the gardener's standpoint is one which promotes the plant's thrifty development, while at the same time its growth respecting time, amount, type and place (localization in plant part) is such as to suit his material purpose. When the fact of the plant's plasticity is known, it remains essential that the proper means for producing desirable modifications be understood; and furthermore, that the limits of variability, which if exceeded will endanger the plant as a whole, be respected.

Summary

LETTUCE EXPERIMENTS.—1. The top-root ratio varied with experimental conditions between the extremes of 0.79 (table IV, lot 1) and 12.96 (table III, lot 7).

2. Under the following circumstances the top-root ratio was increased: Application of superphosphate either alone or with raw sulphur on a fertile fine sandy loam soil (table I); progressive improvement of fertility in a very poor medium sandy loam soil (table II); with an acid muck-and-sand soil mixture as compared with a fertile fine sandy loam, neutral in reaction (table III); the addition of both lime and superphosphate to an acid muck-and-sand soil mixture (table III); shortened period of daylight illumination in comparison with either normal or artificially lengthened day (tables IV and V); use of sodium nitrate alone and with superphosphate in conjunction with either long or short-day light treatment (tables IV and V).

3. The top-root ratio was lowered by long-day illumination (tables IV and V).

4. The coefficients of correlation for the separate lots of plants vary widely and show no definite relation, respecting their magnitude, to average

sizes of whole plants, but on the whole are decidedly positive in character. The coefficient of correlation for all lettuce plants taken together as a single group (975 individuals) is 0.521 ± 0.018.

RADISH EXPERIMENTS.—5. The top-root ratio was higher for plants grown on a soil composed of 5 parts fine sandy loam and 1 part medium coarse sand than for those on soil of same components but with the proportion reversed (table VI).

6. As with lettuce, the top-root ratio was higher for short-day plants and lower for long-day plants in comparison with normal-day plants (table VII).

7. Correlation between top and root was variable in degree but clearly positive in character. The coefficient for all radish plants grouped together (277 individuals) is 0.728 ± 0.019.

TOMATO EXPERIMENTS.—8. The ratios, whether based on the mass relation of whole top, leaves only, or stem only to the root, increased as the size of the container used was greater (table VIII).

9. Higher ratios obtained consistently (though the differences are not mathematically certain) for unpruned plants, for non-deflorated plants both pruned and unpruned, and for plants provided with bottom heat (tables IX, X).

While none of the differences between the coefficients of correlation for the several experimental lots is great enough to be mathematically significant, it is noteworthy that the coefficients are apparently higher for unpruned than pruned plants, for plants from which flowers were removed than those not treated so, for plants grown in larger containers, and also higher as between root and stem than between root and either whole top or only the leaves. The coefficients are more consistent than for either the lettuce or radish plants and the positive nature of correlation even more evident. The coefficient of correlation with all tomato plants thrown into one group is for whole top and root (440 individuals) 0.885 ± 0.007; stem and root (389 individuals) 0.792 ± 0.013; leaves and root (389 individuals) 0.836 ± 0.010.

GENERAL.—The concrete results that have been presented are not altogether new and different. In the main, they confirm other investigations in showing the great variability possible in the mass relation of top to root, and give credence to the idea of an "ideal balance" obtainable, within limits, by nature in the process of natural plant adaptation and by plant growers to suit their needs and purposes. It is thought, however, that improved methods for the analysis of data of this type, especially use of the geometric mean and the coefficient of correlation, have been employed, and furthermore, that the fact of persistent positive correlation in size of top and root, regardless of the wide variation shown under special conditions, has been demonstrated.

MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN.

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THE MECHANISM OF THE WATER TIGHT DOOR OF THE *UTRICULARIA* TRAP

FRANCIS E. LLOYD

(WITH FIVE FIGURES AND ONE PLATE)

Introduction

Of the various devices found among plants with the function of entrapping animals, none has been more intriguing than that of *Utricularia*. Interesting and complex as are the pitchers of *Sarracenia, Darlingtonia, Nepenthes, Cephalotus,* these are passive in their behavior, aside from the secretion of digestive enzymes [HEPBURN and his co-workers (**10**)]. Far more remarkable are considered the submersed *Aldrovandia vesiculosa* L. and the land form *Dionaea muscipula,* the leaves of which, when stimulated, respond by closing over the unwary insect or other animal which touches the sensitive hairs (**3, 6**). *Utricularia,* however, possesses bladder-like traps which are purely mechanical in their action when once set, and entrap small animals in much the same way as does a mouse trap of the old-fashioned kind. The setting, which is automatic, is the result of a peculiar physiological behavior of the walls of the bladder (recently studied by CZAJA). Beyond this, the trapping action is mechanical, the captured animals being hopelessly ensconced within, when death and digestion finally overtake them. As will be seen, the setting of this trap results from negative pressure of water within. The maintenance of this reduced pressure is made possible by the tight closure of the door. Many attempts have been made to explain how this efficiency is attained and it was the unsatisfactory answers to the question which prompted the present inquiry.

The structure of the *Utricularia* trap (or bladder, as it is frequently called) is so well known and has been so often described, that repetition is unnecessary here (see **1**). There are numbers of species, which differ in various details, but they have in common the trap structure, and all appear to work in the same way (**8, 9, 11**). In the present paper attention is confined to *Utricularia minor.* In this species the trap is compressed pear-shaped, with the entrance at the narrow part where it is truncated. The entrance is guarded by a crescentic ridge, the threshold (plate II, fig. 1), against which rests the edge of the door. The hinge of the door is a semicircular fold attached to the walls of the trap from the inner edge of the threshold on one side where it gives on the wall of the bladder about the opening to the corresponding point on the other side. The structure of the door is such that, when its cells are alive and turgid, it has strong outward

spring, but is kept from springing too far forward by its own shape and emplacement (fig. 1). If cut away from the sides of the trap, it springs so far forward that the entrance is entirely open. The capacity of the door for bending resides in the form of the component cells (plate II, fig. 7), which have been often described. There is a vestibule-like space in front of the door, from the inner surfaces of which extend glandular trichomes. Similar trichomes, but of more varied forms, grow out also from the outer surface of the door; and in addition to these there are (usually) four stiff, pointed trichomes which serve as levers, and may be appropriately called trigger hairs (fig. 1). These are attached obliquely to the outer surface of the door near the middle point of the lower free edge. The part of the door

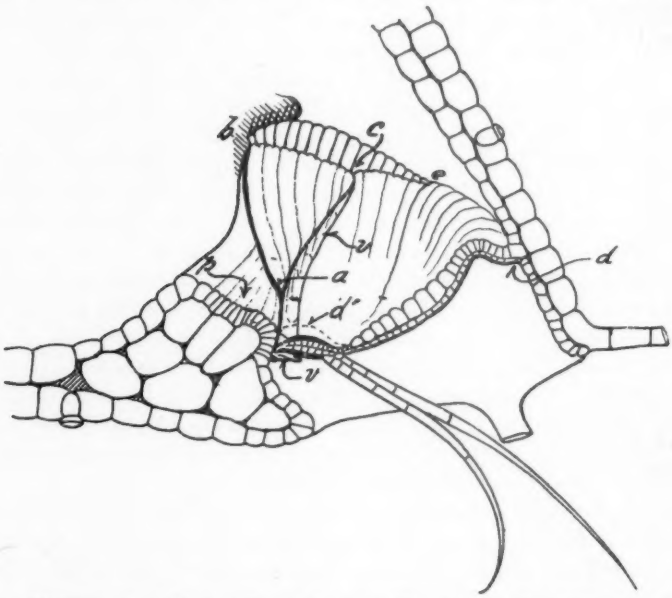

FIG. 1. Diagram of sagittal section and perspective view of the entrance of a trap with the door in the set position and showing the articulation of the door with the wall.

d e. Transverse reach.
b e. Lateral reach.
b c. Articulation with the threshold.
b a. Lateral reach of the free edge of the door, crossing the threshold.
a d'. Middle reach of the door lying in front of the threshold.
The dotted line below d', shows the tripping position of the door.
v. The veil or valve.
p. The pad.

between the insertion of the trigger hairs and the free edge is well thickened (fig. 1) and has a much thickened cuticle (**12**); the trigger hairs and the thickened door edge are so firmly of a piece that when the hairs are bent, the door edge bends with them. The fulcrum for this lever movement is furnished by a very thin spot in the door, just above the insertion of the trigger hairs. It must further be understood that the door is domed, the convex surface being directed outwardly. It is thus able to withstand a considerable, but unmeasured, pressure of water. This pressure results from the circumstance that the water within the bladder is pumped out by the walls, the lateral of which consequently become incurved, as it were collapsed, when the trap thus becomes set. It is to be especially remarked that the set condition is permanent (**12, 6, 13**), from which it must be concluded either that the door does not allow any leakage of water into the interior, or that the cells of the walls can pump it out as rapidly as it gets in.

The foregoing statement of the case is common knowledge and may be summarized briefly as presently follows.

(a) The normal, that is, undamaged or not otherwise discommoded trap, is, when set, water tight as respects mass movement of water (**12, 6**). For only a brief period (less than 1/16 sec.) can water enter. This occurs when the trap is sprung.

(b) The set condition results from the withdrawal of water from the interior of the bladder or trap, through the walls. There is thus set up a reduced pressure of water within. In this condition the lateral expanses of the wall of the trap are strongly inbent, so that they become markedly concave (**2, 12, 6, 13**).

(c) When in this condition, the concave sides of the trap can act as relatively powerful springs. By their sudden bulging out, they can pull through the entrance a considerable column of water with great suddenness. Any animal not too large, which happens to lie within this column of water, is immediately carried into the trap.

(d) The sudden bulging out can occur normally only if the trap is sprung (**12, 6**). This happens when the trigger hairs are suitably disturbed, namely, in such fashion that the middle portion of the free edge of the door is lifted. At the moment of lifting, the side walls spring out, the whole mechanism passing instantly from a state of labile into one of stable equilibrium.[1]

(e) The walls hereupon begin to pump out the water at such a rate that the trap may become set again after the lapse of 15 minutes (**12**) or usually

[1] Springing of the traps occurs when the plant is raised out of the water into the air and the water allowed to drain off. BROCHER noticed a clicking sound caused thereby. MERL'S explanation that the surface tension of the films of water about the trigger hairs accomplishes the tripping is probably correct.

a rather longer period (**13**). We (LLOYD and R. D. GIBBS) have observed the capture of a second worm 35 minutes after the first capture. As soon as a sufficient volume of water has been pumped out, that is, when the sides of the trap have become more or less concave, the trap has become automatically reset.

(f) The pumping out of the water is a function of the walls, which CZAJA described as semipermeable. The action is referred to the two- and four-armed trichomes, which are regarded as absorbent structures (**12, 6**). There is insufficient proof of this, but the matter does not enter into the present discussion.

(g) As stated in (a) above, the set condition is permanent. MERL saw that no dyes enter even during a period of days. CZAJA showed, by passing a human hair underneath the edge of the door, that unless the opening at the entrance is completely closed, the trap does not reset itself.

(h) This hermetically sealed condition, to use MERL's phrase, is owing, according to all observers except BROCHER and WITHYCOMBE, to the firm pressure of the lower free edge of the door against the top of the threshold. According to the majority view the effective sealing of the entrance results from the pressure, eventually produced by the turgor of the door cells, of the free edge of the door against the opposed surface, but seconded by the presence of mucus (**13**) or mucilage which acts as a plugging material, filling the reentrant angle formed at the edge of the door. All students of the matter have agreed on this latter point. The various views with regard to the articulation of door edge with the threshold are indicated by the following passages: ". . . ihr freies Ende auf einem hufeisenförmigen Rahmen als Widerlager ruht" (**8,** 1889, p. 152); ". . . mit der freien vierten Seite dem den Antennen gegenüberliegenden dicken, mit ebenfalls Schleim bildenden Zellen besetzen Mündungsrande von unten d.h. vom Blaseninnern her, anliegend" (**4,** 1888, p. LIX); "ihre Klappe schliesst mittels eines Schleimwulstes so fest, dass aus dem Innern nichts heraustreten kann" (**11,** 1910, p. 211). "Die höchst elastische Klappe die . . . dem Widerlager ziemlich fest aufliegt . . ." (**12,** 1922, p. 73). "Der mittlere Teil liegt dem Polster auf und ist schmal, die seitlichen Teile liegen ebenfalls auf . . ." (**6,** 1922, p. 713); BROCHER, sensing the unsatisfactory character of these or similar descriptions, remarked "s'il était disposé aussi simplement que cela est representé généralement dans les livres, cet état de pression negativ ne pourrait exister, et se maintenir, à l'interieur de l'utricle. Mais tel n'est pas le cas. La disposition est un peu différente et rend cet état de chose possible." He then states that the inward flexing of the sides has the effect of augmenting the curvature of the door in such fashion that "le bord libre de celui-ci s'applique d'autant plus exactement contre le re-

bord (X in his figure) de l'utricle qui lui est opposé, que la courbure de l'opercule est plus considerable'' (**2**, p. 43–44). ''This margin (of the valve or door) is sharp, thin and smooth and rests on the edge of a rim or collar which projects into the interior of the bladder. The collar *obstructs any outward movement* (italics mine) with the result that the valve (door) can only open inwards'' (ARBER, 1920, p. 93). The last quotation is given, not in criticism of its author, but because it summarizes the then accepted view.

WITHYCOMBE (**13**), however, became still more aware of the discrepancy between the accepted accounts and the behavior, and came to believe that the edge of the door rests, not on the top of the threshold, but in a ''slight groove'' in front of the ''collar cells.'' His evidence, however, derived from fixed and paraffin-sectioned material, misled him on this point, the threshold tissues having shrunken so much as to completely distort the shape of the upper surface. As will be shown, the door edge rests against the top of the ''collar cells,'' or pad, and not against their forwardly directed lateral faces. WITHYCOMBE did not apparently apprehend the fact that the door edge crosses the threshold (**6**). The sealing ''mucus'' he supposed to have been secreted by the middle layer of the ''collar cells'' (his figure 5, **13**). WITHYCOMBE, therefore, believed rightly that the door edge presses inwardly against an outwardly opposing surface, but failed to determine what surface, and further accepted mucilage or mucus as the sealing material.

Having thus been led to investigate the matter, it has been possible to show that the mechanism which prevents the inward leakage of water into

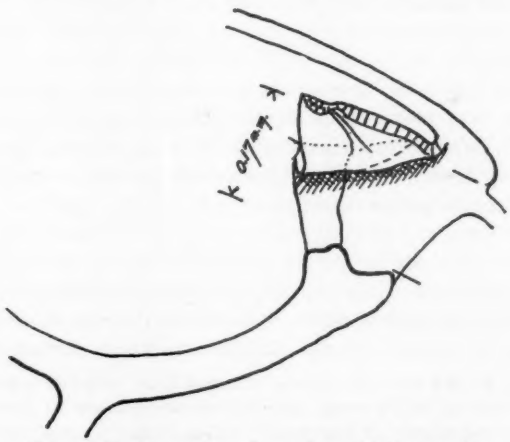

FIG. 2. Position of the door when widely opened.

the trap is not the door alone with a seal of mucilage, but the door acting in cooperation with a valvular membrane attached to the front margin of the threshold. Furthermore, the lower edge of the door lies, when in normal position, not on the threshold, nor pressing outwardly against the outer raised margin, but *pressing inwardly against the outer aspect of this margin.* It is this emplacement of the free edge of the door along a *ligne d'appui,* coupled with the valvular action of *a curtain-like membrane, that together prevent the seepage of water.* The permanence of the set condition of the trap, depending on the foregoing, has been verified by me, with the cooperation of Mr. R. D. GIBBS. In addition we have also determined the behavior of the traps, finding ourselves in agreement with CZAJA and MERL especially in regard to the springing of the trap. We have done this with a needle point and have watched animals (small worms) being entrapped,[2] and have come to the conclusion that the downward movement of the trigger hairs (that is, away from the plane of the antennae) is the most effective, if not the only normal condition for the release of the door. I find that unless the lower edge of the door is engaged by pressing inwardly against the raised outer margin of the threshold (figs. 1, 4), it is unable to resist the pressure of the outer water (assuming the trap to be set) and accordingly bends inwardly, admitting water, or air if water is not present, as BROCHER observed. To release the door edge from this engagement, it must be lifted free of the opposing margin of the threshold. The moment this is accomplished the door gives way entirely to the inrushing water. It is now in order to present an account of the mechanism as thus comprehended. The significance of a number of details of structure hitherto negligible, or at any rate neglected, will now come to light.

Observations

First we will take up the opening into the trap and the threshold. Considering, for the purpose of description, that the antennae are attached to the *upper* lip of the trap, the threshold bars the entrance a short distance within the vestibule. The plane in which it lies will be used as a plane of reference in the following description.

THE THRESHOLD

The threshold is a massive curved structure, the upper surface of which forms a flattened crescent with the horns projecting slightly toward the interior (figs. 1, 4; plate II, figs. 1, 2, 9). Looked at from above, the eye

[2] A motion picture has been made of this, using no artificial assistance, depending wholly on the behavior of the worm. This picture was exhibited at the New York meeting of the Botanical Society of America, December, 1928.

of the observer being in the threshold-plane, the forward or outward margin is curved like a conventional Cupid's bow, the chief curve, that of the body of the bow being in the middle, the rear or inner margin, straight or nearly so. The inner surface extends steeply downwards and is clothed with bifid glandular or absorbing hairs, whatever this may mean. There appears, however, to be another margin within this one just described. This is due to the presence of a pad or mat of epithelium composed of glandular trichomes (**13**) (trapezoidal as seen from above) which in a transverse section of the threshold appear columnar with rounded free surfaces. The shape of the pad is such that the rear limb is symmetrical with the forward limb, being broad near the horns and in the middle (though here not as wide as the total width of the threshold) and narrow between these points (plate II, fig. 9). The relation of this pad of tissue to the threshold as a whole is important and may best be understood by viewing a transverse section of the threshold (sagittal with reference to the trap as a whole). In such a section (fig. 5; plate II, figs. 3–5, 8) one sees the pad as a dense tissue forming the top of the threshold and bending over the outer margin. This bent-over part furnishes a surface for the free edge of the middle portion of the door to rest against when in a set position. As above stated, this margin is outwardly curved. To this curve fits the curve of the middle portion of the free edge of the door when in the set position. It may be observed that the raised outer marginal epithelium with its pad of glandular cells is supported by very large cells jutting upwards above the general level of their neighbors.

The pad ("Pflasterepithel" as GOEBEL called it) is of further and prime importance, in that *the cuticle of its glandular cells exfoliates* during development, and before the trap opens for business, *but remains attached by its forward margin.* This loosened cuticle thus forms a veil suspended between the horns of the threshold and attached to it along a line well below its forward margin at the forward limit of the pad. The presence of this veil has been hitherto overlooked; indeed, I have overlooked it myself for years. It is only with great care that sections can be cut (of fresh material, of course) without tearing it away. When it remains, it is so diaphanous that either it is not seen (in transverse sections of the threshold usually) or appears merely as the top of the threshold when seen looking along the sagittal axis. The forwardly directed surface of the pad and the veil form a pocket. It *thus comes to have a definite function as a valve, sealing the fissure between the edge of the door and the threshold,* just as a bit of leather stops the leakage of the piston head of a pump.

The exfoliation of the veil takes place rather late in the development of the trap. In bladders as small as 0.33 mm. in length it has not yet appeared

but is recognizable at 0.47 mm. At these stages of development the door overhangs the threshold (GOEBEL) coming into its definitive position later. When first evident, it is to be observed over the outer one or two rows of pad cells, the exfoliation evidently beginning in this region and extending back (plate II, figs. 3–5, 8). In younger traps the veil appears more definite and circumscribed, but with age becomes more extensive and ill-defined at its loose posterior border. The ragged appearance of the loose margin of the veil is due to its irregular tearing away from the pad cells in the more interior region, that toward the inner margin of the threshold. The very thin ragged portion bears the evident imprint of the tops of the cells from which it arose.

A feature of the development of the veil must be especially mentioned, namely, that during the earlier period of its exfoliation there is an expansion of its area, so that it balloons away from the cells from which it arises. The impress of the cell top is, as a result, an exaggeration of the pattern present in a surface view of the pad. The large size of the meshes of the veil appears out of congruity with the smallness of the pad cells; it is only when the behavior during origin is known that the incongruity is removed.

The veil is cutinized (is insoluble in strong sulphuric acid) and yellows with iodine, when it can be easily seen. When bleached of iodine and lying in glycerin it becomes exceedingly diaphanous.

THE DOOR

The door, called by some the valve, is a structure of complex curvatures. It may best be described in relation to figures 1 and 3. Figure 3 is introduced for the further purpose of indicating my dissent from CZAJA'S mapping of the areas which he recognizes. His effort was concentrated on explaining the behavior of the door as a structure which presses down and outwardly on the threshold. Since this is not what happens, his description fails in cogency.

The insertion of the fixed margin of the door is along a U-shaped line, the plane of which is almost at right angles (*ca.* 100°) to the threshold plane, and so disposed that the free ends of the U coincide with the ends of the threshold where it gives on the side wall (fig. 1, *b c*). From this line of insertion the door hangs in such fashion that the free edge, that which articulates with the threshold, is nearly circular. This shape is retained normally by its own turgor. Plate II, fig. 6, is a photograph taken at right angles to the plane embracing the free edge of a freshly removed door. When in position, the thin edged sector (*a b c*, *a′b′c′*, fig. 3) is somewhat bent inwardly in adjusting itself to the threshold. In addition to this curvature, the door is also curved normally to the plane of the free edge. When

forced by pressure into a nearly flat plane, it takes the form of fig. 7, plate II. There are, it is evident, as indicated in fig. 3, three regions—a middle generally circular area, truncated below to form the middle region of the free edge (*a a'*), and flanked by two triangular smaller pieces (*a b c, a'b'c'*) which fold along the lines *a c* and *a'c'* when the door is in position. The door is attached along *b c d c'b'*, the hinge at *d* being a strong curve which

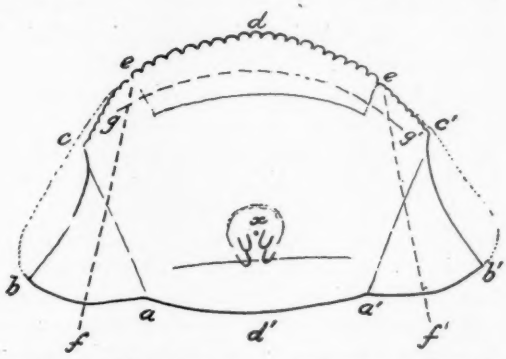

FIG. 3. Map of the door as seen when approximately flat.

b a a' b'. The free edge of the door.
a a'. Middle reach.
ab and a'b'. Lateral reaches.
ac and a'c'. Lines of the fold.
c a a' c'. Follow the curve of the threshold when the door is in the natural set position.
e f and e' f'. Approximate lines of lateral flexure.
g g'. Approximate line of transverse flexure. Median flexure occurs along the line d d'.
χ. Central hinge area; the base of the four trigger hairs are indicated, below which is the thickened margin or wale of the door.
b c and b' c'. Articulations of the door with the threshold.
c e and c' e'. Continuations of the lateral articulations against the wall of the trap.
e d e'. Transverse articulation.

is maintained in the transverse reach *g g'* to become in the lateral reach (*e c, e'c'*) a simple attachment. The structure of the wing *a b c* is continuous with that of the middle piece, and I am unable to identify the triangular areas plotted by CZAJA (*e f g* in his fig. 3, 1922, my fig. 3). The line of the bend, with which CZAJA seems to identify his triangle *e f g*, lies strictly along *a c, a'c'* in my fig. 3 (*e d* in his figure). When in position this line of bending follows the forward edge of the threshold from the horn (*c, c'*, fig. 4) to a point on this edge where the free edge of the door crosses (*a, a'*, figs. 1, 4) to the outer surface of the threshold. The chink formed between the

fold of the door and the edge of the threshold lies behind the veil, which accordingly blocks it. CZAJA'S account postulates a bend running above the thickened margin of the middle piece (*f f′ e′* in his figure), whereas as a matter of fact there is none there. He accords a corresponding discontinuity of structure indicated in his figure by lines. The fact seems rather to be that the structure of the lateral areas is completely congruous with that of the middle piece.

The narrow region below the insertion of the trigger hairs is much thickened and has a thick cuticle. The shape, as seen in section transverse to the run of the edge, is lenticular, the edge which rests on the outer surface of the threshold being rather sharp. Just at and above the insertion of the trigger hairs the door is very thin. This thin area is nearly circular and constitutes a hinge, allowing the thickened edge of the door to bend up and inwardly so that it can come just above the resisting edge of the threshold. When this condition is established, the door has little resistance and flaps inwardly, permitting an inrush of water. This concluded, it springs back into position, the stiff edge sliding over the pad till it regains its *ligne d'appui*. In doing so it pushes before it the loose veil which automatically comes into position as a valve not only near the horns of the crescentic threshold, as above stated, but along the whole length of the edge of the middle piece as well.

It is difficult, indeed, almost impossible to cut a section of fresh material so that the door remains in exactly the right position. A half-door inevitably curves free of the edge and lies more or less above the threshold, the veil then looking like a ragged mass of stuff not calculated to attract attention. I have accordingly studied the silhouettes of entire fresh traps making negatives with long exposures. These, when examined, usually show a shadow made by the veil, and the door in its proper position. Once one knows what to look for, it is quite possible, often easy, to see the veil in the living trap by looking obliquely into the entrance with a low power objective. In some cases even the quasi-cellular structure can be seen (plate II, figs. 1, 2, 10).

Little has been said yet about the hinge of the door as a whole. In the transverse reach this hinge is a strong flexure toward the inside of the trap, bending back again into the curvature of the middle piece. As the transverse reach fades into the lateral reaches, the bend of the hinge becomes less pronounced, until it merely curves downwards and a little away from the wall. In the region over the end of the threshold a mass of tissue lifts the end of the door (marked *b c*, *b′ c′*, fig. 1) away somewhat from the wall, a group of large cells, a sort of buttress, causing this (*c*, *c′*, fig. 1, 4). This grades in the forward direction into the wall and offers attachment to the

stretch of the arch b to e, fig. 1. At point e there occurs a slight angle, breaking a little the continuity of the arch, the point at which the strongly bent hinge cells (e d e') begin.

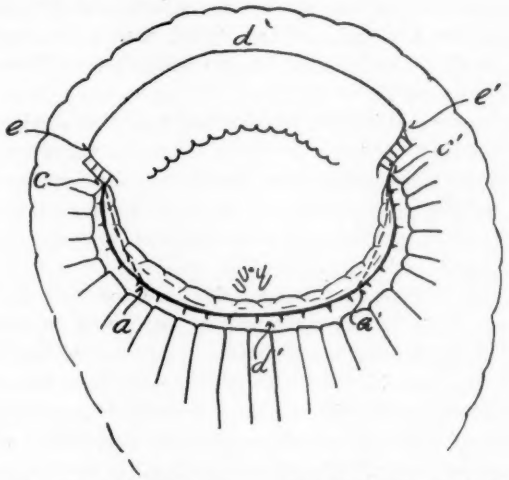

FIG. 4. Diagram of the entrance from the inside of the trap.

The behavior of these regions of the door along the arch of attachment may be seen by studying the silhouette of a trap which has swallowed a glass rod (fig. 2). A trap freed from the plant will, when the end of the glass rod is pushed against the trigger hairs, jump forward and swallow the end of the rod, as CZAJA found using a needle. I found that a rod 0.1 mm. in diameter is easily swallowed. If the rod is tapering, it may be pushed forward, with precautions to do no damage to the entrance mechanism of the trap, till the door is opened to its maximum. When in this position the rod was 0.17 mm. in diameter, representing approximately the maximum possible displacement of the door, probably more than normally occurs. The middle piece of the door (f, e, e' f', fig. 3) suffers maximum displacement, the sides of the door being bent upwards approximately along the lines e f and $e'f'$, fig. 3. This bend is most pronounced toward f, and towards e gradually fades into the curvature between e and e', though not without a jog at these points. The tissues along $e-f$ and $e'f'$ are thus strongly pulled out of equilibrium and tend to straighten back in a direction normal to $e-f$ and $e-f'$. The cells at right angles to the line $g-g'$ tend to bend back normally to this line. It is certain that the cells along b c e and $b'c'e'$ are displaced from position only very slightly, merely enough to accommodate themselves to the sharp upward bend along e f and $e'f'$.

What the maximum displacement of the door is when it opens by displacement by the water column pressing against it cannot precisely be said, but, since (in the trap I measured) the maximum opening has the transverse diameter of 0.17 mm. and the same trap immediately, on springing the door, swallowed a rod 0.1 mm. in diameter, it must open more widely than has been supposed. MERL speaks of a lunate "halbmondförmig" slit. Any opening short of a good-sized throat could not engulf the large objects often found in the traps; and it must not be forgotten that during the time the door is open, less than 1/16 of a second, a column of water fully 1 mm. long passes in. During the movement of this column the door must remain stationary in a position presenting a throat of diameter greater than 0.1 mm. To do this the sides of the door must be bent upwards, approximately, though not as much, as in fig. 7.

The recovery of the set position of the door does not wait on the complete relaxation of the lateral walls, and in fact probably always closes before. This is shown by the fact that, after any event of tripping, the walls will on puncturing the bladder with a sharp needle relax further to the maximum possible extent (**12**). The fact is used here to emphasize the vigorous action of the door, which recovers its position in spite of a still inflowing column of water. The change in shape of the trap after tripping with a needle and after puncturing with a needle was illustrated by MERL.

Flexure of the door also occurs along the line $d\ d'$ in the sense opposite to the flexure $e\ f$ and $e'f'$. The great thickness and elasticity of the door

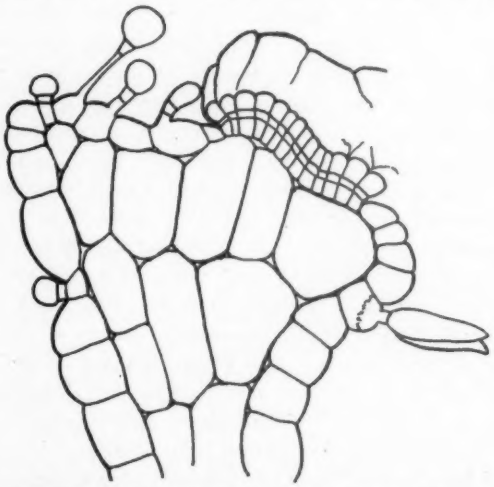

FIG. 5. Transverse section of the threshold showing the veil when fully developed.

edge, forming a wale of tissue reaching from a to a', enable it, while bending before the moving column of water, on release of pressure to immediately resume its semicircular form, bringing it back into its original position, the whole edge from b to b' (fig. 3) being in the form of an unbroken arch (plate II, fig. 6). This wale of tissue is important, therefore, in bringing the door into the set position. Its initial flexure, due to the leverage by the trigger hairs, is at its middle point (d'). The pressure of the water causes it to arch in the opposite sense, the flexure spreading outwardly along the radii from the centre χ.

Correlated with the remarkable degree of flexure possible, and with the strong resistance of the door to deformation, is the form of the cell walls. These are never straight, but curved like so many springs. This is true of both radial and periclinal walls (plate II, fig. 7). This is, of course, a matter of common observation. What is important for the correct understanding of the working of the door is the proper evaluation of the rôle of the thickened wale along the middle reach of the door edge. This acts by swinging into position, engaging the outer edge of the threshold as a catch which in spite of its firm engagement can be easily released by downward pressure of the trigger hairs. Its almost cartilaginous consistency is due in large part to the much thickened outer walls (MERL) of the very small cells.

Summary

It has been shown in the foregoing that when the trap of *Utricularia minor* is in the set position, the middle part of the free edge of the door rests against the outer surface of the pad of glandular cells of the threshold, and not, as heretofore supposed, against the top or any part of the top or against the edge of the pad. It maintains its position against water pressure by virtue of the resistance offered by the threshold. The outer reaches of the door edge cross obliquely the sides of the threshold. There is of mechanical necessity, therefore, a fold between the middle and lateral portions of the door which would, if not otherwise provided against, allow inflow of water under the reduced pressure known to be present in the trap when set. This possible leakage of water into the interior of the trap is prevented by the presence of a veil or curtain attached to the outer surface of the threshold and stretching from one horn to the other of the crescentic threshold in such a manner as to act as a valve to exclude water by pressing on the edge and folds of the door. This veil, or valve, is the outer cuticle released from the cells of the pad, being set free from the inner margin of the pad and remaining firmly attached in front. The veil shows the imprint of the top of the cells from which it sprung. The earlier formed part of the veil suffers considerable extension, or ballooning, recalling the

behavior of the cuticle in certain other glandular trichomes (*Pelargonium*). The veil arises during the later period of development of the trap, so that when the trap begins to function as such, the veil is ready in position.

McGill University,
Montreal, Canada.

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EXPLANATION OF PLATE

FIG. 1. Entrance of trap viewed from the outside showing the veil and the upper surface of the threshold. The door is sprung outwardly by its own turgor, but it is so seen because a part of it has been removed in sectioning the trap.

FIG. 2. The veil and front edge of the threshold of another trap. The lower edge of the door can be seen again in the sprung position.

FIG. 3. Section of the threshold of a young trap showing the exfoliating cuticle at a rather early stage.

FIG. 4. A somewhat later condition.

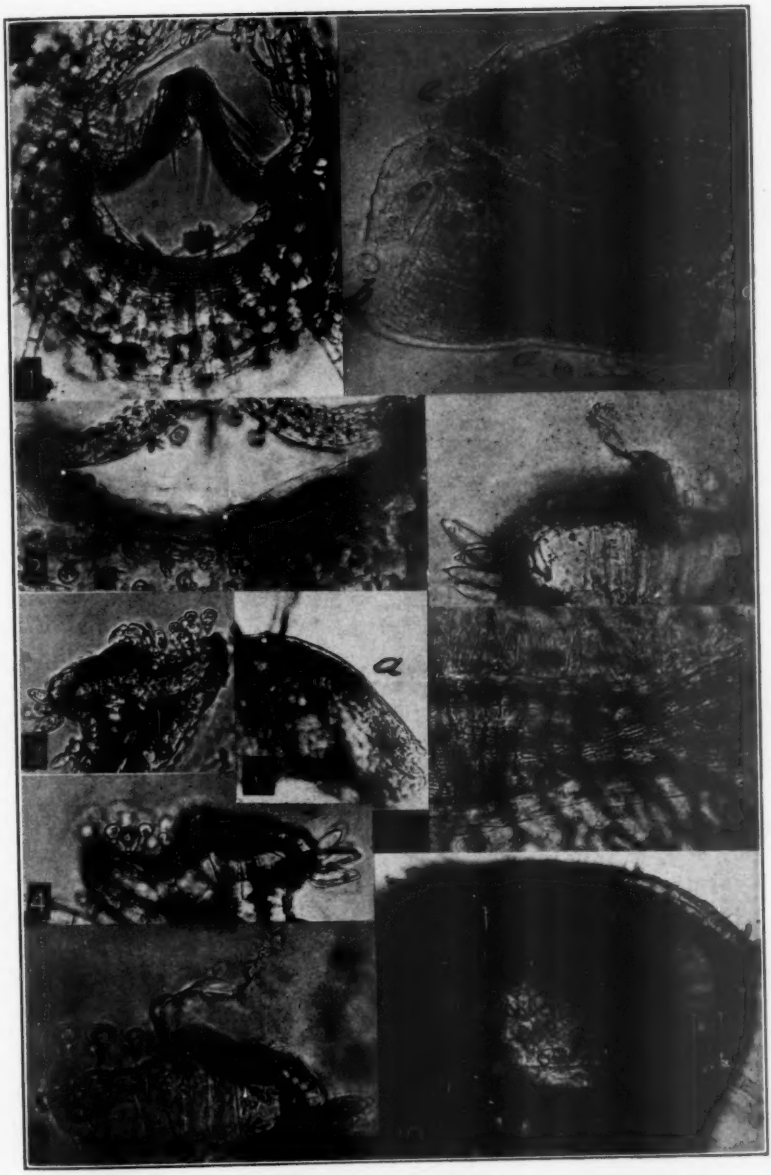
FIGS. 5 and 8. The fully developed veil as seen in transverse section of the threshold.

FIG. 6. Half of the door showing the thick wale above the middle reach of the lower edge and the thin lateral portion which crosses the threshold from *a* to *b*—text fig. 1.

FIG. 7. A half-door laid out nearly flat viewing the inside surface. Letters as in text fig. 1.

FIG. 9. View of a little over half a threshold seen from above. The anterior margin bears the veil. The inside face of the threshold (above) bears glandular trichomes. The trapezoidal cells of the pad can be seen. The dark patches are caused by air in the intercellular spaces, here as in other figures.

FIG. 10. View of the door and veil in the set position as seen when looking into the threshold of a living set trap.



LLOYD–UTRICULARIA

SOME CRITICAL COMMENTS ON THE METHODS EMPLOYED IN THE EXPRESSION OF LEAF SAPS[1]

BERNARD S. MEYER

(WITH ONE FIGURE)

In many types of plant physiological research, accurate determinations of the physico-chemical properties of expressed plant saps are important. In undertaking such determinations, however, the technical methods of collecting and treating the samples and of expressing the saps have not always been judiciously chosen to meet the requirements of the investigation. Such methods have sometimes been followed according to blind prescription with little or no regard for the limitations imposed upon them by the physiology or anatomy of the tissues. The following comments upon the technique of such determinations, and the attempted critical evaluation of the more generally used methods are based upon the experience gained in an extended investigation upon the physical and chemical properties of expressed leaf saps. The discussion in this paper refers primarily, therefore, to leaves and leaf saps.

The earliest investigators of the properties of expressed plant tissue fluids carried out their determinations upon saps expressed from living, untreated tissues. ANDRÉ (**1**) appears to have been the first to observe that fractions of sap successively expressed from an untreated leaf sample showed progressively increasing concentrations of solutes as measured by the depression of the freezing point.

DIXON and ATKINS (**2**) obtained results similar to those of ANDRÉ when they expressed sap from untreated plant tissues. They explained the progressive increase in the osmotic concentrations of successively expressed fractions of sap by assuming that a larger number of cells burst at the higher pressures, thus permitting solutes to escape to which the protoplasmic membranes of the unbroken cells at the low pressures were impermeable.

Since this differential expression of sap from untreated tissues makes it practically impossible to express from them representative samples of sap, DIXON and ATKINS introduced the method of freezing the leaves in liquid air before subjecting them to pressure. They presented data to show that the successively expressed fractions of sap from a leaf sample frozen in liquid air did not differ materially in osmotic value. They also found that the volume of sap expressed from frozen leaves was larger than could be expressed from unfrozen leaves, and was freer from debris.

[1] Papers from the Department of Botany, the Ohio State University, no. 221.

GORTNER and HARRIS (**5**), also principally interested in determinations of the osmotic values of expressed plant tissue fluids, substituted the ice-salt bath as a more universally available mode of freezing. In this method the tissues to be frozen are placed in a glass tube of suitable size, which is then stoppered and immersed in an ice-salt bath in which a temperature of $-15°$ C. to $-20°$ C. is usually maintained. The usual field precautions to be observed with this freezing method are discussed by its originators. A number of other investigators have employed this method.

GORTNER, LAWRENCE, and HARRIS (**6**) checked the conclusion of DIXON and ATKINS that the concentration of solutes in samples of sap successively expressed from unfrozen leaf samples showed a progressive increase. They expressed the sap from untreated leaf samples from a number of species of plants and found some in which successively expressed portions of the sap showed decreasing concentrations of solutes, and others in which all of the fractions of expressed sap showed approximately the same concentrations of solutes. Although their results deviate from those obtained by DIXON and ATKINS, they do not invalidate the principle that sap samples expressed from unfrozen tissues cannot be taken as representative of the conditions existing in the tissue from which they have been exposed.

Methods of freezing leaf samples in solid carbon dioxide have been employed by HARVEY (**7**), LEWIS and TUTTLE (**10**), MEYER (**11**), and others.

GAIL (**3**) ground the leaves of western conifers through a fine meat grinder before expressing the sap. Some samples were also frozen but GAIL reported that there was little difference between the osmotic value of the samples merely ground, and those which were both ground and frozen.

NEWTON, BROWN, and MARTIN (**12**) have recently described a method of finely grinding plant tissues and expressing the fluids at low pressure which they believe results in the expression of a sap which represents substantially the composition of the original tissue fluids. All operations were carried out at a temperature close to 0° C. Since the object of this method is to obtain the cell contents in as nearly unchanged conditions as possible, freezing of the tissue is not permissible because of its known precipitating effect upon proteins. This method promises to prove of value and general application in indicating the distribution of any constituent of plant tissues between the physiologically active and the inert portions of the tissues.

GOLDSMITH and SMITH (**4**) appear to have been the first to rely upon the method of treating leaf samples with a toxic vapor in an extensive investigation of the physico-chemical properties of plant saps. In a recent investigation they used chloroform vapor treatments upon the leaves of the Engelmann spruce (*Picea engelmanni*) with apparently satisfactory results. The leaves were exposed to the vapor for thirty-six hours before the saps were

expressed, the investigators relying upon refrigeration during this period to prevent any appreciable enzyme action. Freezing point depressions obtained with saps expressed after this treatment were corrected for the solubility of chloroform in water. This method was checked against the ice-salt freezing method for leaves in the summer condition and excellent agreement found in the results, but apparently it was not checked against other methods while the leaves were in the winter condition. The subsequent discussion in this paper shows that such a check would have been desirable. However, there is no indication in the results obtained that this method is not effective with winter-hardened leaf tissues.

During the past several years the writer has made a number of comparative studies of the effects of several methods of treating leaf samples upon the amount of sap which can be expressed from them and the osmotic value of the sap. Parallel series of determinations were made at different seasons for the leaves of several evergreens in the living condition, after freezing in an ice-salt bath, after freezing in solid carbon dioxide, and after grinding to a shredded pulp.

Table I records the results of a representative series of determinations upon the 1927 leaves of the pitch pine (*Pinus rigida*). Similar results were obtained in every other series of determinations made.

TABLE I

EFFECT OF VARIOUS TREATMENTS OF PITCH PINE LEAVES UPON THE AMOUNT OF SAP WHICH CAN BE EXPRESSED, AND UPON THE OSMOTIC VALUE OF THE EXPRESSED SAP
PRESSURE = 5,000 LBS. PER SQ. IN.

DATE	MOISTURE CONTENT	DETERMINATION	UNTREATED	FROZEN (ICE-SALT)	FROZEN (CO_2)	GROUND
	per cent.					
Aug. 3, 1927	59.4	Sap expressed (cc. per 100 gm.)	23.9	44.3	47.6	42.9
		Osmotic value (atmospheres)	9.40	15.20	15.91	18.68
Jan. 22, 1928	54.7	Sap expressed (cc. per 100 gm.)	17.7	15.0	41.0	31.3
		Osmotic value (atmospheres)	12.41	13.04	21.12	25.73

An analysis of these data brings out a number of facts which are pertinent to the present discussion. The water content of the leaves was 4.7 per cent. higher in August than in January, a fact which may be expected to have a minor influence on the volumes of sap which could be expressed.

In the summer approximately the same volumes of sap could be expressed from samples frozen in an ice-salt bath, from samples frozen in

solid carbon dioxide, and from samples shredded through a meat grinder. A much larger volume of sap could be expressed from all the treated samples than from the untreated sample.

The summer osmotic value of the sap expressed from the ground sample was about three atmospheres higher than the osmotic value of the sap expressed from either of the frozen samples, which checked closely with each other. A much higher osmotic value was obtained with all the treated samples than with the untreated sample. The higher value shown by the sap from the ground sample was perhaps due to the favorable conditions for oxidations and other chemical changes which probably occur in the tissues during and immediately following the grinding process.

In the winter, only from the sample frozen in solid carbon dioxide was a volume of sap expressed which compared with the volumes expressed during the summer from all the treated samples. The volume of sap expressed from leaves frozen in an ice-salt bath was of the same order of magnitude as the volume expressed from the untreated leaves. The volume expressed from the ground leaves was distinctly smaller than the volume expressed from leaf samples frozen in solid carbon dioxide.

The winter osmotic value of the sap expressed from the leaves frozen in an ice-salt bath was not appreciably different from that of the sap expressed from unfrozen leaves. The sap expressed from the leaf sample frozen in solid carbon dioxide showed a much higher value, and that expressed from the ground leaf tissue was still higher, but the last two values were obviously of the same order of magnitude.

It is believed that the difference between the results obtained with leaves in the summer condition, and the results obtained with leaves in the winter condition is due principally to an increase in the amount of "bound water" in the leaf tissues during the winter months. In a previous paper (**11**), the probable relationship between seasonal variations in the bound water content, and seasonal variations in the hydrophilic colloid content of the leaves of this species has been indicated. During the summer months it appears that only a relatively small amount of the water present in the tissues of pitch pine leaves is bound. The methods of freezing in an ice-salt bath, freezing in solid carbon dioxide, or grinding, are therefore all effective in increasing membrane permeability, presumably through disorganization of the protoplasm, and thus permit the expression of a representative sample of sap.

During the winter months, however, a relatively large proportion of the water present in the tissues apparently is in the bound condition. The ice-salt freezing method does not have a drastic enough effect upon the tissues to permit the expression of a representative sample of sap. Presumably

this is because the molecular forces binding the water are of sufficient magnitude to prevent the occurrence of any appreciable amount of crystallization of water in the tissues. At this temperature, according to all present conceptions of the mechanism of ice crystallization in plant tissues, crystals ordinarily form only in the intercellular spaces, withdrawing water from the cells as they elongate. The sap expressed is therefore similar in amount and properties to that expressed from untreated leaves.

At the temperature of solid carbon dioxide (−80° C., and lower), the forces of crystallization are undoubtedly of sufficient magnitude to overcome the molecular forces binding water. Leaves are usually frozen solid by this treatment, indicating that most of the water present in the tissues freezes within the cells. The result of this treatment is that representative samples of sap can be expressed under pressure.

The grinding treatment also permits the expression of representative samples of sap at all seasons of the year. The effectiveness of this treatment is apparently due to the disruption of the physical relationship existing between the bound water and the tissues, through the bruising and tearing action which the grinding process exerts on them.

The writer (**11**) has already called attention to the discrepancy between the results obtained by GAIL (**3**) and those obtained by KORSTIAN (**9**) in their investigations of the winter osmotic values of the expressed leaf saps of certain western evergreens. The divergent results obtained by these two investigators are apparently due to different methods of treating the leaf samples. KORSTIAN froze the leaves in an ice-salt bath while GAIL, as has already been noted, used what was essentially a grinding treatment.

The demonstrated and theoretically expected limitation of the ice-salt freezing method to unhardened tissues makes it desirable that a field method be devised which is not subject to such a limitation. The liquid air freezing method, vapor treatment methods, and grinding method are difficult to adapt to ordinary field conditions. Neither is the spraying method of freezing with solid carbon dioxide, as usually employed, feasible in the field.

Recent developments in the commercial production of solid carbon dioxide[2] have permitted the development of a field method of freezing leaf samples with solid carbon dioxide which will be briefly described.

The solid carbon dioxide must first be reduced to fragments varying up to the size of a small marble. These crushed fragments are poured into an insulated container[3] until a layer about one inch in thickness is deposited

[2] Solid carbon dioxide is manufactured and sold by the Dry Ice Corporation of America, New York and Chicago, under the trade name of "Dry-Ice."

[3] Stanley "Super-Vac" Food Jars (all steel) in the quart size have been found to be very serviceable for this purpose.

in the bottom. A stoppered tube of suitable size (about 8 inches long and 1.25 inches in diameter) is inserted and held upright while the carbon dioxide is poured around it until the jar is filled to a level slightly below the top of the tube. The jar is stoppered and is then ready to be carried into the field.

Leaves are thrust as rapidly as collected into a glass tube of the same size as the one inserted into the jar. When filled the tube is stoppered and substituted for the empty tube in the jar. This particular procedure is necessary because the solid carbon dioxide so rapidly becomes compacted in the jar, that it becomes practically impossible to dislodge the individual particles sufficiently to force the collecting tube into the mass of solid carbon dioxide. The solid carbon dioxide slowly disappears by sublimation, but usually a sufficient amount remains in the jar to keep the leaf samples frozen for about 24 hours.

There is no reason to believe that the method of freezing leaf samples in liquid air as first employed by DIXON and ATKINS will lead to results for sap determinations which are markedly different from those obtained with the carbon dioxide freezing treatment. Although the writer has not had the experimental opportunity to confirm this statement, comparative data from HARVEY (**7**) support it. For hardened cabbage leaves HARVEY recorded a difference of only 2.3 atmospheres in osmotic value between sap samples expressed after these two treatments. For unhardened cabbage leaves he recorded a difference of 1.7 atmospheres.

KNUDSON and GINSBERG (**8**) found no appreciable differences in the osmotic values of the saps expressed from two samples of the leaves of an unhardened greenhouse plant, *Iresine herbstii,* one frozen in an ice-salt bath, the other in liquid air. HARVEY recorded an osmotic value about 6.5 atmospheres higher for saps expressed from unhardened cabbage leaves after freezing in liquid air than for saps expressed from unhardened cabbage leaves frozen at $-5°$ C. It is probable, however, that $-5°$ C. is not a low enough temperature to have an effect on unhardened cabbage leaves comparable with the effect of an ice-salt bath on the leaves of *Iresine.*

The press diagrammed in text figure 1 was devised especially for the expression of leaf saps, and has been found in practice to give excellent service for this purpose. This press involves certain principles in design which apparently have not been previously employed, and has some advantages over other styles of presses in common use. The principal parts are the piston *A,* and the cylinder *B,* which were turned from steel and ground to fit within 0.001 inch. The dimensions are shown in the figure. The groove *a* cut around the inside wall of the cylinder one inch from the top is designed to prevent the upward movement of sap past that point. The groove *b,*

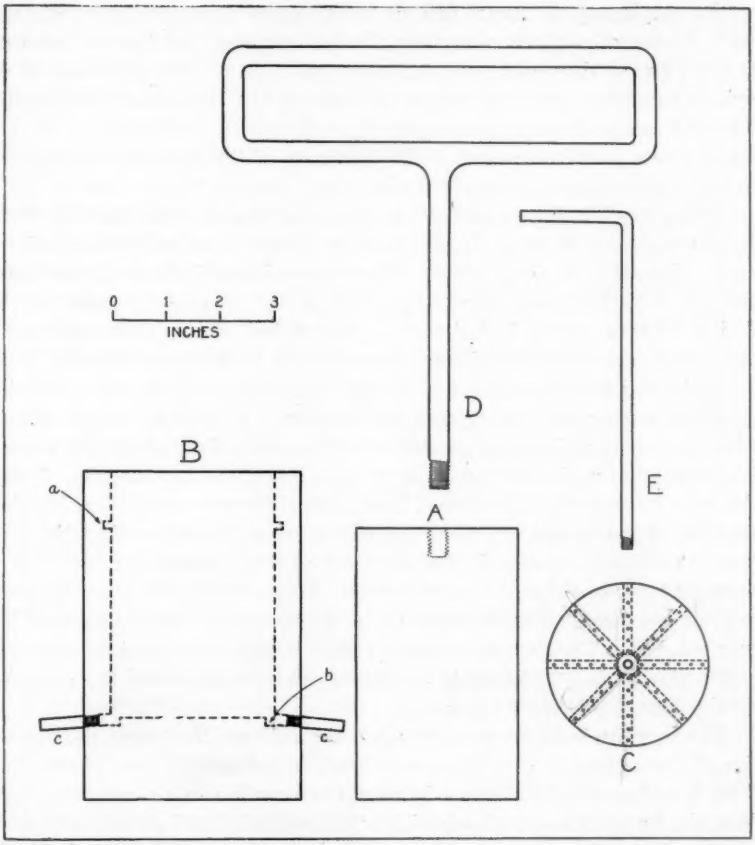


FIG. 1. Press for the expression of leaf tissue fluids. *A*, piston; *B*, cylinder; *C*, perforated disk; *D*, handle for piston; *E*, handle for disk; *a*, groove for preventing upward movement of sap; *b*, groove for collecting sap; *cc*, delivery tubes.

cut around the base of the cylinder, adjacent to the vertical wall, is designed to collect the sap and feed it to the two delivery tubes, *cc*, on opposite sides of the cylinder. These tubes are made of brass and threaded into place so they can easily be removed for cleaning. An important auxiliary part of this press is the metal disk *C*. On the lower side of this disk are eight equally spaced radiating grooves. Six one-sixteenth inch holes, spaced at equal intervals, were drilled through the metal disk into each groove. The removal of both the piston and the disk from the cylinder is facilitated by means of the metal handles, *D* and *E*, which screw into place.

By employing the metal disk described above it is possible to dispense with the use of cloth for wrapping the leaf samples. It is only necessary to place two or three disks of linen cloth, cut to fit the circumference of the cylinder snugly, on top of the metal disk in the bottom of the cylinder. The leaf sample is then placed directly on top of the cloth disks. In fact, when leaves containing considerable amounts of ligneous tissues are used, there is no need even for the disks of cloth.

This press has given satisfactory results for the expression of leaf fluids from a variety of plants. It is convenient in that it is readily handled and easily cleaned. A press of the dimensions shown in text figure 1 will accommodate leaf samples weighing up to 100 grams. Presses of this design may, of course, be built of any convenient size or, if deemed advisable, other metals than steel could be employed in their construction.

Following the suggestion of KNUDSON and GINSBERG (8) the writer has employed a materials testing machine as a source of known pressure for use with this press, with entirely satisfactory results. In packing the press it is necessary to avoid any bunching or wadding of the leaf sample. Unless the leaves are evenly distributed throughout the press cylinder, bunched portions of the sample will be subjected to a greater pressure than the more loosely packed portions. In careful work such an unequal distribution of pressure must be reduced to a minimum. It is necessary to allow the press to drain for several minutes after the maximum pressure to be employed has been reached. The most satisfactory results appear to be attained when the pressure is applied just rapidly enough to cause the expressed sap to trickle slowly from the delivery tubes.

The magnitude of the pressure used will have an effect upon the properties of the expressed sap. KNUDSON and GINSBERG (8) have shown that there is an important difference between the osmotic value of saps expressed from the leaves of *Iresine herbstii* at a pressure of 10,000 pounds and those expressed at a pressure of 50,000 pounds, both for samples frozen in liquid air and for samples frozen in an ice-salt bath. The pressure employed should depend upon the structural character of the plant organ, and upon the purpose for which the expressed fluid is to be used.

It is highly desirable that, before investigations involving determinations of the physico-chemical properties of expressed plant saps are undertaken, preliminary studies should first be conducted to determine the most suitable method of treating the tissue, and the proper magnitude of the pressure to be applied. Papers reporting the results of any such investigation should include the following information: water content of the tissue, exact treatment and method of handling the tissue, magnitude of the pressure employed, and the volume of sap expressed as a percentage of the fresh

weight or water content of the tissue. Only with such information available can the reader reconstruct for himself a reasonably accurate picture of the exact effects of the treatment and pressure upon the tissue, and only if such information be supplied can the determinations be duplicated.

Summary

1. A summary of the more important methods which have been used in the treatment of leaves before the expression of leaf saps is presented. The discussion attempts to evaluate these methods with especial regard to the limitations and advantages of each.

2. A field method of freezing leaf samples in solid carbon dioxide is described.

3. Details are given for the construction of a press which embodies certain principles in design which do not appear to have been previously used. This type of press has been found to give satisfactory results for the expression of saps from the leaves of a variety of species of plants.

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CHEMICAL RELATIONSHIP BETWEEN SCION AND STOCK IN CITRUS[1]

A. R. C. Haas and F. F. Halma

(WITH ONE FIGURE)

It is commonly known that *Citrus* varieties are propagated commercially by budding. The most important varieties grown in California are Washington Navel orange and Valencia orange (*Citrus sinensis* Osbeck), Marsh grapefruit (*Citrus maxima* Merrill), and Eureka and Lisbon lemon (*Citrus limonia* Osbeck). The rootstocks upon which these varieties are budded are sweet orange (*Citrus sinensis* Osbeck), sour orange (*Citrus aurantium* L.), grapefruit (*Citrus maxima* Merrill), and to a small extent rough lemon (*Citrus limonia*) and trifoliate orange (*Poncirus trifoliata* Raf.). The degree of congeniality shown between scion and rootstock in Citrus has been reported by Webber (7), Newman (6), and others. Their observations show in a general way that the lemon scion overgrows the sour rootstock as is indicated by a bulge just above the bud union, and overgrows the sweet stock to a less extent, whereas the opposite effect is seen when the lemon is budded on grapefruit or trifoliate orange, in which case the trunk of the rootstock is larger than the lemon trunk. Similar but less pronounced size differences exist when the Navel or Valencia orange or the Marsh grapefruit is budded on sour, sweet or trifoliate rootstocks. On the other hand a smooth bud union results when these standard varieties are grown on rough-lemon rootstock. In general the degree of so-called congeniality or compatibility implies a comparison of the relative rate of growth of scion and stock; the more nearly equal the rate of growth of scion and stock the greater the congeniality or compatibility.

No adequate data are available from which to formulate a hypothesis which may lead to an understanding of the relationship of scion and stock. Limited data by Jensen and others (5) indicate that in the case of lemon on sour stock, the amount of starch is greater above the bud union than in the case of lemon on grapefruit stock. This, however, may be considered effect rather than cause, since the overgrowth in the former case has an effect comparable to that of girdling. Our knowledge of the organic constituents of evergreen trees is scanty, and although carbohydrates may be fairly accurately determined, yet their amounts are subject to considerable fluctuation not only throughout the year but also throughout the day. On the other hand the inorganic constituents of mature parts of Citrus trees

[1] Paper no. 205, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

fluctuate very little and hence studies along this line are more promising. For this reason we have studied the inorganic composition of scion and stock in Citrus.

Before reporting the results of the present investigation it is necessary to refer to previous work which shows that fundamental physical and chemical differences exist in the leaves of varieties of Citrus that are ordinarily combined in budding. The ash and calcium of the water-soluble fraction of mature normal Washington Navel and Valencia orange leaves exceed those of Eureka lemon (HAAS, **1**). The sap of normal mature lemon leaves is less active osmotically and contains less ash and calcium but more magnesium than the sap of orange leaves (HAAS and HALMA, **2**). Investigations on the sap and the morphology of leaves of these two Citrus species indicate that the lemon leaf has a more efficient photosynthetic system than the orange leaf (HALMA and HAAS, **3**; HALMA, **4**). Furthermore, it is a matter of common observation that the growth rate of unbudded seedlings or cuttings of lemon is greater than that of sour orange, sweet orange or grapefruit, but about equal to that of rough lemon. It seems reasonable to assume that varieties or species that differ in so many respects must undergo some changes in their physical or chemical make-up when combined with one another by means of budding. The data presented herewith show that a chemical difference exists between scion and stock. It appears that the scion species or variety influences the amount of soluble magnesium in the bark of the stock.

The material analyzed consisted entirely of bark. In all cases the cambium was active so that it was an easy matter to remove the bark. Care was taken to collect material only from trees growing on definitely known stocks. The bark was obtained from areas immediately above and below the bud union. The portion of the tree from which the material was taken was carefully cleaned; the bark was peeled off and dried in an oven at 65° C. The dried material was finely pulverized so as to pass through a 20-mesh sieve. Water-soluble and insoluble ash, calcium and magnesium were determined. The analytical procedure was essentially the same as that previously used (**1**).

Previous studies (**1**) have shown that the leaves of sweet orange and lemon could be distinguished by the water solubility of the calcium in the dried leaves: the dry matter of the sweet orange leaves contained about two and one-half times as much water-soluble calcium as that of the lemon leaves. It was assumed in the present investigation that possibly a similar chemical difference may exist in the bark. It was further assumed that, if this were found to be the case, it might serve as a means for identifying the variety by means of bark analysis.

From table I, however, it is clear that this calcium relationship does not exist in the bark, where the water solubility is extremely low as compared with that of the leaves. It may be of interest to add that the total calcium as a percentage of the dry matter is about equal to that found in the leaves.

Table I suggests that the amount of soluble magnesium as a percentage of the dry matter or of soluble ash depends upon the position which the variety occupies in the budded tree; for where the sweet orange served as the scion, its soluble magnesium content was considerably higher than when it served as a stock for the lemon scion. It is also clear from table I that sour, sweet and grapefruit stocks gave values similar to that of the lemon scion.

Since the material in table I was considered inadequate to definitely establish this magnesium relationship between scion and stock, a sufficiently large number of bark samples representing various commonly occurring combinations of scion and stock were collected. These samples were obtained from trees varying in age from seven to thirty-five years and growing under widely different soil and climatic conditions in southern California. A limited number of samples were also obtained from unbudded trees. Because of the possible influence of seasonal variations, the material was collected both in March and in August of the same year. These samples were analyzed by the same procedure as was used for the previous material.

The data given in table II are so grouped that the magnesium relationship between scion and stock of the same tree may very readily be seen. Figure 1 gives the average values and enables one to summarize the data readily. If we consider the trees having a lemon scion we find that the average values for the soluble magnesium as a percentage of the dry matter are rather uniform both in the scion and stock, regardless of the four varieties of stock used. On the other hand if the scion is Valencia orange or Marsh grapefruit the values for the soluble magnesium are considerably higher, not only in the scion but also in the stock, which in this case includes both sweet and sour orange. The same type of sour orange which served as a stock for the lemon scion shows a higher soluble magnesium content when, instead of a lemon scion, it had a Marsh grapefruit or Valencia orange scion.

Turning to the unbudded trees where there is no scion influence, we find that the bark of sour orange and rough lemon is appreciably lower in soluble magnesium than that of either sweet orange or grapefruit. The values for unbudded sour and rough-lemon seedlings are not changed appreciably by budding a lemon scion on them. However, the value for

TABLE I

INORGANIC COMPOSITION OF BARK OF DIFFERENT VARIETIES OF CITRUS SCIONS AND STOCKS EXPRESSED AS PERCENTAGES. (MATERIAL COLLECTED OCTOBER, 1927)

SAMPLE NO.	MATERIAL	TOTAL ASH IN DRY MATTER	SOLUBLE ASH IN		TOTAL CALCIUM IN		SOLUBLE CALCIUM IN			TOTAL MAGNESIUM IN		SOLUBLE MAGNESIUM IN		
			TOTAL ASH	DRY MATTER	TOTAL ASH	DRY MATTER	DRY MATTER	SOLUBLE ASH	TOTAL CALCIUM	TOTAL ASH	DRY MATTER	DRY MATTER	SOLUBLE ASH	TOTAL MAGNESIUM
17	Eureka lemon scion (Sour stock)	14.38	9.23	1.33	36.45	5.24	0.12	6.13	1.55	2.02	0.29	0.048	3.60	16.46
18	Sour stock of above tree	14.88	7.47	1.11	36.85	5.48	0.08	7.43	1.51	0.75	0.11	0.051	4.61	46.00
19	Eureka lemon scion (Sour stock)	14.69	5.81	0.85	37.96	5.58	0.11	12.32	1.89	1.31	0.19	0.038	4.41	19.54
20	Sour stock of above tree	15.34	6.99	1.07	37.61	5.77	0.09	8.45	1.57	1.31	0.20	0.044	4.15	22.13
21	Eureka lemon scion (Sour stock)	14.89	8.08	1.20	36.77	4.52	0.09	7.59	2.01	1.44	0.18	0.035	3.13	21.13
22	Sour stock of above tree	12.36	9.69	1.20	36.56	5.48	0.09	7.74	1.70	1.22	0.18	0.038	2.94	19.38
2	Sour stock (Eureka lemon scion)	12.77	10.51	1.34	35.65	4.55	0.13	9.49	2.80	1.29	0.16	0.054	4.01	32.76
3	" " " " "	13.48	8.89	1.20	35.48	4.78	0.13	10.57	2.65	1.04	0.14	0.051	4.29	36.59
6	" " " " "	12.56	9.75	1.23	35.69	4.48	0.14	11.09	3.03	0.90	0.11	0.041	3.35	36.36
7	" " " " "	13.58	8.51	1.16	35.60	4.83	0.11	9.40	2.25	0.94	0.13	0.045	3.89	35.42
8	" " " " "	13.66	8.83	1.21	35.45	4.84	0.14	11.97	2.98	0.70	0.10	0.037	3.05	38.24
15	" " " " "	10.75	6.08	0.94	36.77	5.72	0.10	10.91	1.80	0.66	0.10	0.031	3.30	30.23
23	" " " " "	7.03	8.41	1.20	37.09	5.27	0.24	19.85	4.50	1.00	0.14	0.053	4.39	36.84
13	Sweet " " " "	18.31	7.29	1.33	32.82	6.01	0.16	11.73	2.61	0.70	0.13	0.043	3.18	35.19
14	" " " " "	16.56	9.22	1.53	36.02	5.97	0.21	13.57	3.47	0.72	0.12	0.058	3.78	48.21
12	Grapefruit stock (Eureka lemon scion)	18.81	8.66	1.63	30.10	5.66	0.17	10.57	3.04	1.00	0.19	0.057	3.52	30.38
16	" " " " "	17.15	7.28	1.25	35.58	6.10	0.12	9.67	1.98	0.72	0.12	0.032	2.60	26.09
1	Valencia scion (Sweet stock)	13.70	10.31	1.41	35.62	4.88	0.19	13.66	3.95	1.36	0.19	0.072	5.12	38.89
5	" " " " "	13.31	11.79	1.57	35.20	4.68	0.24	15.28	5.12	1.39	0.18	0.095	6.08	51.56
10	" " " " "	12.94	11.49	1.48	35.91	4.65	0.22	14.58	4.67	1.03	0.13	0.084	5.66	63.46
4	" " " " "	11.02	13.12	1.45	34.67	3.82	0.16	11.23	4.60	1.95	0.21	0.084	5.81	39.19
9	" " " " "	10.54	12.75	1.34	34.15	3.60	0.15	11.21	4.19	1.76	0.19	0.073	5.42	39.19
11	" " " " "	10.86	12.12	1.31	34.52	3.75	0.14	11.01	3.86	1.61	0.17	0.071	5.39	40.68
24	" " " " "	6.77	10.18	1.50	34.06	5.03	0.22	14.69	4.39	1.69	0.24	0.100	6.78	42.86

TABLE II

INORGANIC COMPOSITION OF BARK OF DIFFERENT VARIETIES OF CITRUS SCIONS AND STOCKS EXPRESSED AS PERCENTAGES

SAMPLE NO.	DATE COLLECTED 1928	MATERIAL	TOTAL ASH IN DRY MATTER	SOLUBLE ASH IN		TOTAL CALCIUM IN		SOLUBLE CALCIUM IN			TOTAL MAGNESIUM IN		SOLUBLE MAGNESIUM IN		
				TOTAL ASH	DRY MATTER	TOTAL ASH	DRY MATTER	DRY MATTER	SOLUBLE ASH	TOTAL CALCIUM	TOTAL ASH	DRY MATTER	DRY MATTER	SOLUBLE ASH	TOTAL MAGNESIUM
9	March	Eureka lemon scion (sour stock)	11.51	10.29	1.18	35.82	4.12	0.11	9.29	2.67	0.36	0.04	0.020	1.69	47.62
10	"	Sour stock of above tree	12.65	7.97	1.01	37.79	4.78	0.13	13.29	2.80	0.68	0.09	0.034	3.37	39.53
12	"	Lisbon lemon scion (sour stock)	13.41	9.41	1.43	35.60	4.78	0.09	6.31	1.88	0.75	0.10	0.032	2.24	32.00
13	"	Sour stock of above tree	13.51	9.67	1.31	36.33	4.91	0.13	10.00	2.66	0.57	0.08	0.032	2.45	41.38
5	"	Sour seedling (unbudded)	13.43	9.78	1.30	34.94	4.69	0.10	7.48	2.07	0.70	0.09	0.024	2.49	34.29
12	August	" " "	12.04	10.08	1.21	37.94	4.57	0.09	7.08	1.88	0.95	0.11	0.020	1.65	17.54
13	"	" " "	15.09	5.38	0.81	39.12	5.90	0.10	12.07	1.66	0.78	0.12	0.034	4.19	28.81
18	March	Eureka lemon scion (rough lemon stock)	12.35	8.45	1.04	36.52	4.51	0.08	7.28	1.69	0.52	0.06	0.020	1.72	28.12
19	"	Rough lemon stock of above tree	12.26	8.16	1.52	36.26	6.76	0.11	6.92	1.56	0.40	0.07	0.026	1.73	35.71
22	"	Eureka lemon scion (rough lemon stock)	11.97	8.99	1.08	36.49	4.37	0.08	7.43	1.83	0.62	0.07	0.022	2.04	29.73
23	"	Rough lemon stock of above tree	19.21	8.61	1.65	36.14	6.94	0.11	6.47	1.54	0.40	0.08	0.039	2.38	51.85
11	August	Rough lemon seedling (unbudded)	16.85	8.49	1.43	39.01	6.57	0.10	6.85	1.49	0.54	0.09	0.034	2.38	37.78
48	"	" " "	13.11	9.90	1.30	37.49	4.92	0.12	8.94	2.36	0.99	0.13	0.038	2.93	29.23
6	March	Eureka lemon scion (sweet stock)	12.42	10.56	1.31	34.50	4.28	0.08	6.41	1.96	0.66	0.08	0.030	2.25	36.11
7	"	Sweet stock of above tree	15.45	9.77	1.51	34.36	5.31	0.15	9.80	2.79	1.05	0.16	0.044	2.91	27.16
32	"	Eureka lemon scion (sweet stock)	11.90	11.36	1.35	34.29	4.08	0.07	5.34	1.77	0.59	0.07	0.021	1.55	30.00
33	"	Sweet stock of above tree	14.58	9.00	1.31	35.67	5.20	0.14	10.82	2.73	0.25	0.04	0.032	2.44	88.89
26	"	Lisbon lemon scion (sweet stock)	15.71	5.16	0.81	37.43	5.88	0.09	11.36	1.56	0.55	0.09	0.024	2.96	27.91
27	"	Sweet stock of above tree	16.19	9.52	1.54	37.12	6.01	0.20	12.82	3.29	0.81	0.13	0.059	3.82	44.64
28	"	Lisbon lemon scion (sweet stock)	15.84	5.27	0.84	38.31	6.07	0.09	10.64	1.46	0.49	0.08	0.027	3.19	34.29
7	August	Sweet seedling (unbudded)	16.60	7.71	1.28	38.82	6.45	0.12	12.66	2.51	1.30	0.22	0.070	5.47	32.40
47	"	" " "	15.36	9.06	1.39	37.76	5.80	0.17	12.36	2.97	1.34	0.21	0.070	5.03	33.98
52	"	" " "	15.64	8.30	1.30	38.99	6.10	0.19	14.33	3.05	1.33	0.21	0.070	5.39	33.65
20	March	Lisbon lemon scion (grapefruit stock)	12.13	11.13	1.21	35.87	4.35	0.11	8.15	2.35	0.41	0.05	0.022	1.63	44.00
21	"	Grapefruit stock of above tree	15.19	11.18	1.70	35.88	5.45	0.19	11.43	3.56	0.54	0.08	0.034	2.00	41.46
24	"	Lisbon lemon scion (grapefruit stock)	10.10	13.88	1.40	34.03	3.44	0.08	6.00	2.45	0.95	0.09	0.033	2.33	34.14
25	"	Grapefruit stock of above tree	13.09	13.88	1.82	35.03	4.58	0.21	11.55	4.57	1.06	0.14	0.054	3.03	39.53

TABLE II—(*Concluded*)

INORGANIC COMPOSITION OF BARK OF DIFFERENT VARIETIES OF CITRUS SCIONS AND STOCKS EXPRESSED AS PERCENTAGES

SAMPLE NO.	DATE COLLECTED 1928	MATERIAL	TOTAL ASH IN DRY MATTER	SOLUBLE ASH IN		TOTAL CALCIUM IN		SOLUBLE CALCIUM IN			TOTAL MAGNESIUM IN		SOLUBLE MAGNESIUM IN		
				TOTAL ASH	DRY MATTER	TOTAL ASH	DRY MATTER	DRY MATTER	SOLUBLE ASH	TOTAL CALCIUM	TOTAL ASH	DRY MATTER	DRY MATTER	SOLUBLE ASH	TOTAL MAGNESIUM
30	March	Lisbon lemon scion (grapefruit stock).	17.73	4.35	0.77	37.22	6.60	0.07	9.85	1.15	0.62	0.11	0.020	2.59	18.18
31	"	Grapefruit stock of above tree.....	15.31	6.65	1.02	37.30	5.71	0.13	12.78	2.28	0.63	0.10	0.030	2.91	30.95
34	"	Eureka lemon scion (grapefruit stock).	9.37	10.21	0.96	35.16	3.30	0.10	10.02	2.91	1.28	0.12	0.031	3.20	25.42
35	"	Grapefruit stock of above tree.....	12.02	9.68	1.16	36.90	4.44	0.14	11.85	3.11	0.88	0.11	0.038	3.26	35.85
14	August	Grapefruit seedling (unbudded).....	17.05	9.16	1.56	38.17	6.51	0.22	14.21	3.41	1.43	0.24	0.086	5.51	35.25
17	"	Eureka lemon scion.....	10.99	9.92	1.09	38.44	4.22	0.13	10.28	2.65	1.29	0.14	0.042	3.85	29.58
4	"	Marsh grapefruit intermediate.....	13.37	8.52	1.14	35.44	4.74	0.13	11.58	2.78	0.73	0.10	0.032	2.81	32.65
8	"	Sweet stock	15.56	8.24	1.28	39.21	6.10	0.13	9.83	2.07	0.78	0.12	0.038	2.96	31.15
20	"	Eureka lemon scion.....	11.47	10.72	1.23	36.17	4.15	0.10	8.29	2.46	1.22	0.14	0.032	2.60	26.67
18	"	Marsh grapefruit intermediate.....	13.92	8.92	1.24	38.05	5.30	0.13	10.47	2.45	0.93	0.13	0.036	2.90	26.87
1	"	Sweet stock	16.17	8.53	1.38	39.14	6.33	0.14	10.00	2.18	0.79	0.13	0.036	2.61	28.12
53	"	Eureka lemon scion.....	10.18	10.96	1.12	37.65	3.83	0.11	10.22	2.97	1.36	0.14	0.030	2.69	21.74
9	"	Marsh grapefruit intermediate.....	13.50	8.83	1.19	37.74	5.10	0.11	9.06	2.12	0.95	0.13	0.030	2.52	23.44
2	"	Sweet stock	15.89	8.21	1.30	38.89	6.18	0.12	8.90	1.88	0.73	0.12	0.036	2.76	31.03
16	"	Marsh grapefruit scion (sweet stock)..	14.26	9.13	1.30	38.34	5.47	0.14	10.91	2.60	1.51	0.22	0.070	5.38	32.40
15	"	Sweet stock of above tree.....	16.07	9.07	1.46	38.72	6.20	0.16	10.29	2.41	1.07	0.17	0.054	3.70	31.40
19	"	Marsh grapefruit scion (sweet stock)..	14.09	9.35	1.32	37.28	5.25	0.16	12.29	3.08	1.60	0.25	0.060	4.55	26.55
22	"	Sweet stock of above tree.....	15.42	8.67	1.34	37.67	5.81	0.17	13.00	2.99	1.15	0.18	0.066	4.93	37.08
6	"	Marsh grapefruit scion (sweet stock)..	14.25	9.39	1.34	38.60	5.50	0.19	14.50	3.53	1.80	0.26	0.094	7.03	36.72
51	"	Sweet stock of above tree.....	16.43	8.40	1.38	40.16	6.60	0.15	10.87	2.27	1.14	0.19	0.062	4.49	32.98
3	March	Marsh grapefruit scion (sour stock)...	12.94	14.04	1.82	33.69	4.36	0.19	10.46	4.36	1.61	0.21	0.089	4.85	42.31
4	"	Sour stock of above tree.....	11.14	12.59	1.40	34.12	3.80	0.15	10.41	3.84	1.72	0.19	0.068	4.85	35.42
14	"	Marsh grapefruit scion (sour stock)...	12.68	14.99	1.90	33.79	4.28	0.21	10.84	4.81	1.28	0.18	0.086	4.53	53.09
15	"	Sour stock of above tree.....	11.56	12.15	1.40	35.50	4.10	0.13	9.26	3.17	1.44	0.17	0.067	4.70	39.76
1	"	Valencia orange scion (sour stock)....	12.39	15.43	1.91	33.08	4.10	0.23	12.03	5.61	1.69	0.21	0.110	5.75	52.38
2	"	Sour stock of above tree.....	10.05	13.57	1.34	34.17	3.43	0.16	11.58	4.60	1.37	0.14	0.056	4.11	40.58
16	"	Valencia orange scion (sour stock)....	12.01	18.39	2.21	33.32	4.00	0.24	11.05	6.10	2.39	0.29	0.140	6.10	47.22
17	"	Sour stock of above tree.....	10.22	15.90	1.63	33.90	3.47	0.16	9.96	4.67	2.27	0.23	0.104	6.40	44.83

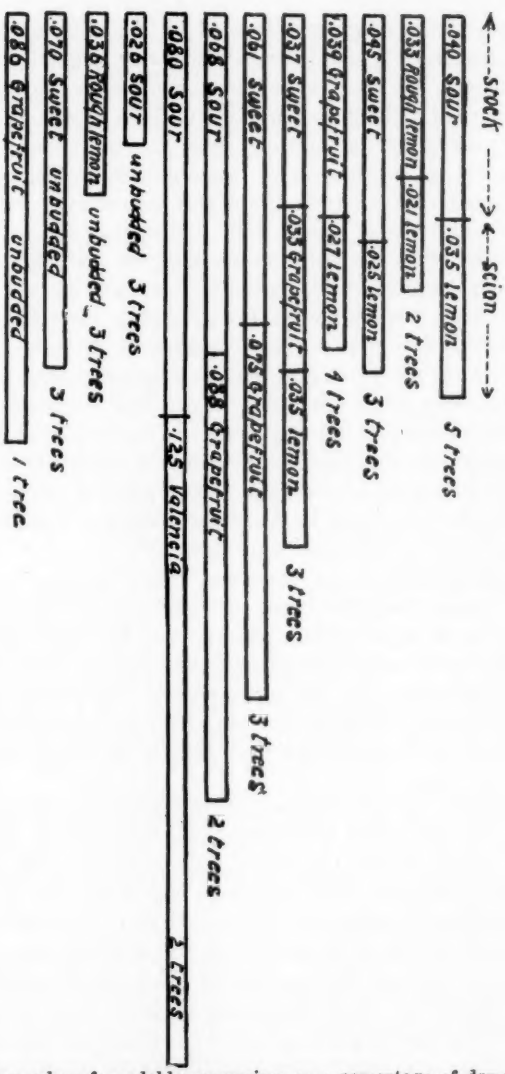

FIG. 1. Average values for soluble magnesium as a percentage of dry matter for stock and scion.

sour orange when used as a stock for either Valencia orange or Marsh grapefruit scions, is increased above the value found for unbudded sour seedlings.

A clear case of the dominance exerted by the scion as far as soluble magnesium is concerned is seen in trees which were originally Marsh grapefruit on sweet stock, but which were later topworked to Eureka lemon. In this case the values for the grapefruit intermediate as well as for the sweet stock are approximately the same as those for the lemon scion. Other trees of the same age growing in the same orchard, which were not topworked, and hence retained their grapefruit top, showed values for both scion and stock which were considerably higher than those found in topworked trees. In other words the replacement of the original grapefruit top by the lemon scion exerted a profound influence on both the original scion and the stock. Therefore, as far as soluble magnesium content is concerned, there seems to be no very marked stock influence upon the lemon scion. On the other hand, as is seen in figure 1, when the grapefruit or sweet orange scion is on sour stock, which when unbudded has a low soluble magnesium content, the value for the stock is greatly increased. But we also see that the unbudded sweet orange and grapefruit have higher values than the unbudded sour orange. Here again we cannot escape the conclusion that the grapefruit or Valencia scion was responsible for the increase in the values found in the sour stock.

It appears from figure 1 that there may be an increase in soluble magnesium as one passes from the lemon scion to the various stocks, but the reverse is true to an even greater extent when the scion is Valencia or grapefruit. The reasons for the influence of scion on stock are unknown but it may be suggested that the differences in the growth rate between lemon and sweet orange or grapefruit may play an important rôle in that the leaves of the former would draw more heavily on the magnesium supply than those of the latter.

There are several points of interest in the tables in addition to the case of soluble magnesium. It is well known that the calcium content of the ash of normal mature Citrus leaves is nearly constant, being about 35 per cent. Approximately the same percentage was found in the ash of all bark samples analyzed, regardless of whether the variety was the scion or the stock. Moreover the dry matter of the lemon leaves contains about 18 per cent. of soluble calcium, whereas that of sweet orange contains approximately 50 per cent. (1). Such differences do not exist in the trunk bark; as a matter of fact, the values as given in the tables are extremely low in comparison and do not reveal any great difference between the different varieties.

Throughout the tables there is a slight suggestion of inorganic gradients in the trunk. Since the samples consisted of bark taken immediately above and below the bud union, one could not expect to find consistent differences

in the same direction. However, in the case of the trees having a lemon scion, a grapefruit intermediate and a sweet stock, the samples necessarily represent greater intervals along the trunk and main branches. Here we find a gradual increase from top to bottom in the soluble and total calcium in the dry matter, but a decrease in the soluble ash and total magnesium in the total ash.

Summary

Some differences in inorganic composition between scion and stock of Citrus were studied. It was found that the soluble magnesium as a percentage either of the dry matter or of the soluble ash of the trunk bark is lowest in the lemon and sour orange and highest in the sweet orange and grapefruit. The values found in the bark of the stock vary according to the scion variety with which it is combined. If the scion is lemon, which normally has a low value, and the stock is sweet orange or grapefruit, the values for the stocks are much lower than they would be if they had not been budded. On the other hand if sweet orange or grapefruit (with a higher value than the lemon) is growing on the sour orange, then the value of the latter is considerably higher than it would be if the lemon were the scion.

CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

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STORAGE OF TRUCK CROPS: THE GIRASOLE, *HELIANTHUS TUBEROSUS*[*][1]

HAMILTON P. TRAUB,[2] CLIFFORD J. THOR, J. J. WILLAMAN, AND R. OLIVER

(WITH FOUR FIGURES)

The girasole,[3] *Helianthus tuberosus*, has had much interest centered upon it since the report of JACKSON, SILSBEE and PROFFITT (**4**) on the possible commercial preparation from it of fructose, a sugar sweeter than sucrose, according to PAUL (**7**), DEERR (**3**), SALE and SKINNER (**8**), BIESTER, WOOD and WAHLIN (**2**) and SPENGLER and TRAEGEL (**10**). A survey of the plants which might serve as a source for the commercial production of fructose shows that the girasole ranks among the more promising. The plant has been in general use in many European countries for a long time as a truck crop and as a stock food, and to a lesser extent this is true for certain sections of the United States.

One of the chief problems in connection with this crop is that of storage. The periderm of the girasole tuber is very thin and easily ruptured. There is a rapid loss of moisture on exposure to atmosphere at room temperature. The tubers are subject to parasitic diseases under ordinary storage conditions.[4] Most authorities seem to be agreed that the best method of storage is the practice of leaving the tubers in the ground and digging them when needed. SHOEMAKER (**9**) reports good success with tubers stored in burlap bags in a cold cellar at Washington, D. C., from November, 1925, to March, 1926, without shriveling or other difficulty. The temperature was kept near the freezing point. The preliminary experiments reported in this paper covering the period from August 30, 1927, to March 22, 1928, were carried out in order to secure more accurate information with regard to the storage of this crop.

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[1] Thanks are due LAWRENCE ZELENY for competent assistance in certain of the chemical determinations.

[2] Chief, Division of Horticulture, Texas Agricultural Experiment Station, College Station, Texas, since April, 1928.

[3] *Helianthus tuberosus* is *not* an artichoke and is *not* native to Palestine; therefore, "Jerusalem artichoke" is a lengthy misnomer, and is being replaced, for purposes of economy, if for no other reason, by the simpler term, "girasole." See L. H. BAILEY, Vegetable Gardening, 1890.

[4] A study of the diseases of girasole tubers in storage was carried on parallel with the experiments reported in this paper by Prof. H. W. JOHNSON, of the Division of Plant Pathology. This report will be published separately.

Methods

The general plan of the experiment included the study of the dry matter and water soluble carbohydrates in the tubers of the Portland variety from August 30, 1927 to March 22, 1928, (1) outdoors on silt loam, (2) outdoors on sandy loam, (3) in common storage at 34°–49.5° F., (4) common storage at 33.5°–44° F., (5) in cold storage at 32°–35° F. The tubers were washed, dried with a towel and ground to a pulp in a food chopper. The pulp was thoroughly mixed, after which 100 gm. were taken for a moisture determination, and two 30-gm. portions for sugar analysis. Fifteen cc. of water were added to each of the 30-gm. samples in a 250-cc. beaker and the mixture was set in a boiling water bath for one hour. The extract was pressed from the pulp while hot, with a small juice press, into a 100-cc. volumetric flask. The pulp was washed with two 15-cc. portions of boiling water, the washings being added to the first extract. The combined extracts were hydrolyzed with 2.5 cc. of 8.12 N HCl on a water bath at 70–80° C., for 35 minutes and cooled to room temperature. To clarify the hydrolyzed extract, solid neutral lead acetate was added until precipitation was complete. A white precipitate of lead chloride is deposited first and this is followed by colloidal matter. The mixture was then diluted to volume (100 cc.) and filtered, deleaded with solid disodium phosphate and filtered again.

Aliquots of the deleaded filtrate, which was clear and practically colorless, were used for the selective determination of fructose and total reducing substances by Ost's cupro-carbonate method as modified by Nyns (**5**). The cupro-carbonate solution was prepared as follows: 250 gm. K_2CO_3 and 100 gm. $KHCO_3$ were dissolved in 700 cc. boiling water; 25.3 gm. of very pure $CuSO_4 \cdot 5H_2O$ dissolved in about 100 cc. of water were added, and the solution was made up to 1 liter. One or 2-cc. aliquots of the clarified sugar solution were placed in 250-cc. Erlenmeyer flasks and distilled water was added to make a total of 20 cc. Then 50 cc. of cupro-carbonate solution were added and the mixture was reduced: one set on a water bath kept at 48.5–49° C. for the determination of fructose, and another set on a boiling water bath for the determination of total water soluble carbohydrates. Reduction was carried out for exactly 2.5 hours, when the cuprous oxide precipitates were collected in Gooch crucibles on asbestos mats. The amount of copper was determined in each case by the permanganate method as given in Official Methods (**1**). These values, referred to tables in Oliver's paper (**6**), gave the amount of fructose and of total reducing sugars. Subtracting the former from the latter gave "glucose," a term here used to include glucose and any other reducing substances other than fructose which may be present.

Kinds of carbohydrates present

In an attempt to determine the amount of free reducing sugars in the girasole tuber, a sample (Nov. 3, 1927) was extracted in the usual way, except that some $CaCO_3$ was added to prevent hydrolysis of polysaccharides by the natural acidity of the juice. Hydrolysis with HCl was omitted but the extract was clarified with neutral lead acetate and deleaded as usual. This unhydrolyzed extract was subjected to the cupro-carbonate reduction at 48.5–49° C., and at 100° C. In both cases no more copper was reduced than in the case of the blank for the method, indicating the absence of free fructose or free "glucose."

Samples of the same material were extracted at room temperature (23° C.) instead of at 100° and in the presence of $CaCO_3$ to prevent hydrolysis. In this case the extract was also filtered to remove $CaCO_3$ particles. The extract was then hydrolyzed with HCl and run through the usual procedure. In this way it was hoped to remove the water soluble levulins or inulides without removing inulin itself. However, the analyses gave only slightly lower values for fructose and "glucose" in this extract than in the case of the extracts prepared in the usual way.

	Fructose *per cent.*	"Glucose" *per cent.*
Usual method	10.90	5.71
Cold extraction in presence of $CaCO_3$ and hydrolysis with HCl	9.68	4.97

This indicates that practically all the water soluble polysaccharides are inulides, and that the inulin content is low, which is in harmony with the work of WILLAMAN (**12**).

Preliminary analyses, 1926–27

The preliminary work was done on the crop of 1926. One variety of white girasole was grown in St. Louis County. Another lot of tubers, of an unknown variety, was secured from Portland, Oregon, and grown at University Farm; and some of the same variety, which had been grown at Portland, were sent to us in October and placed in a root cellar. Some French Mammoth were also grown at University Farm. Samples of tubers were dug at intervals during the winter and spring, some being analyzed immediately and others kept in a root cellar for various periods. The data are presented in table I.

It will be noted that the fructose content is rather low in comparison with that found by JACKSON, SILSBEE and PROFFITT (**4**). This is probably due to immaturity. The sample from Portland, Oregon, contains nearly twice as much fructose as the others.

During the winter, whether the tubers are in the ground or in storage, the fructose-glucose ratio is rather low. By May the ratio has increased

TABLE I

COMPOSITION OF GIRASOLE TUBERS, CROP OF 1926

DATE DUG	STORAGE	FRUCTOSE		GLUCOSE*		FRUCTOSE PLUS GLUCOSE		FRUCTOSE / GLUCOSE
		IN TUBERS	IN EXTRACTABLE DRY MATTER	IN TUBERS	IN EXTRACTABLE DRY MATTER	IN TUBERS	IN EXTRACTABLE DRY MATTER	
		Grown in St. Louis County, Minnesota						
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
October	10 weeks in root cellar	10.08		3.72		13.80		2.71
"	11 weeks in root cellar	7.66		4.75		12.41		1.62
"	12 weeks in root cellar	8.42		4.78		13.20		1.76
"	13 weeks in root cellar	8.72		5.14		13.86		1.69
"	15 weeks in root cellar	8.24		4.56		13.31		1.81
"	16 weeks in root cellar	7.91		5.65		13.56		1.40
		Variety grown at Portland, Oregon						
October	5 months in sand in root cellar	15.47		8.28		23.75		1.86
		Variety from Portland, Oregon, grown at University Farm						
February 23	freshly dug	7.91		5.07		12.98		1.56
March 12	freshly dug	7.88		5.30		13.18		1.48
" 12	6 weeks in root cellar	7.20	41.8	4.94	28.7	12.14	70.5	1.46
" 30	3 weeks in root cellar	7.06		5.66		12.72		1.25
April 9	freshly dug	7.56		5.09		12.65		1.48
" 18	freshly dug	5.64	35.2	5.38	33.6	11.02	68.8	1.04
" 29	freshly dug	6.34	32.5	3.32	17.0	9.66	49.5	1.91
May 25	freshly dug	6.23	39.2	1.80	11.3	8.03	50.5	3.46
		French Mammoth, grown at University Farm						
February 23	1 week in root cellar	10.30		4.37		14.67		2.35
March 14	1 week in root cellar	8.02		4.27		12.29		1.88
" 30	freshly dug	8.45		4.49		12.94		1.88
April 9	freshly dug	7.84		5.50		13.34		1.43
" 9	4 weeks in root cellar	7.23	42.4	4.87	28.6	12.10	71.0	1.48
May 10	freshly dug	6.73	38.8	2.86	16.5	9.59	55.3	2.35
" 25	freshly dug	6.33	41.9	2.00	13.2	8.33	55.1	3.16

* Glucose here means reducing sugars other than fructose in the hydrolyzed extract.

markedly. It must be pointed out that direct comparison may not be made between these 1926 samples and those grown in 1927.

Experiments in 1927–28

The Portland variety of girasole was grown at University Farm in 1927 in sandy loam and in silt loam. They were analyzed when freshly dug on August 30, when the tubers were still immature; on November 3, when they had apparently gained their maximum development; and on December 20, January 30, and March 22, during the storage period. On November 3, some of the tubers on the sandy loam were dug and placed in three types of storage. The conditions of storage and the behavior of the tubers under these conditions is shown in table II and figure 1. The results of the analyses are shown in table III and in figures 2, 3, and 4.

TABLE II

PER CENT. OF SOUND, SHRIVELED AND DISEASED GIRASOLE TUBERS, UNDER DIFFERENT STORAGE CONDITIONS. GROWN AT UNIVERSITY FARM, ST. PAUL, MINNESOTA

STORAGE CONDITIONS		DATE OF INSPECTION	CONDITION OF TUBERS EXPRESSED IN PER CENT. OF ORIGINAL NUMBERS		
TEMPERATURE	RELATIVE HUMIDITY		SOUND	SHRIVELED	DISEASED
	per cent.		*per cent.*	*per cent.*	*per cent.*
Common storage 34–49.5° F.	73.9–86.1	January 30, 1928	27.7	38.7**	33.5
		March 29, 1928	7.5	6.9**	85.5
Common storage 33.5–44° F.	89.3–92.0	January 30, 1928	48.6	33.3**	18.0
		March 29, 1928	47.3	44.6**	19.3
Cold storage 32–35° F.	89.1–92.0	January 30, 1928	85.2	10.0*	4.7
		March 29, 1928	80.5	14.1*	5.2

* Slightly shriveled.
** Much shriveled.

Condition of tubers.—The data in the table indicate that the higher temperatures and lower humidities induce the shrivelling of the tubers as well as the attack by pathogenic organisms. On the other hand, the tubers keep very well at lower temperatures and higher humidities. Under the most favorable conditions supplied in these experiments (32°–35° F., and 89–92 per cent. humidity) 80 per cent. of the tubers were sound at the end of the period, less than 15 per cent. were slightly shrivelled, and 5 per cent. were diseased. Under the least favorable conditions (34°–49° F. and 74–86 per cent. humidity) only 7 per cent. remained sound, 6 per cent. were shrivelled and 85 per cent. were diseased.

3.16 | 55.1 | 8.33 | 13.2 | 2.00 | 41.9 | 6.33 | freshly dug | 25
* Glucose here means reducing sugars other than fructose in the hydrolyzed extract.

TABLE III

SEASONAL VARIATION IN MOISTURE, DRY MATTER, FRUCTOSE AND TOTAL WATER SOLUBLE CARBOHYDRATES IN GIRASOLE TUBERS, PORTLAND VARIETY, IN THE GROUND AND IN STORAGE. GROWN AT UNIVERSITY FARM, ST. PAUL, MINNESOTA

STORAGE CONDITIONS	DATE	GREEN WEIGHT BASIS					DRY WEIGHT BASIS			RATIOS	
		MOISTURE	DRY MATTER	FRUCTOSE	GLUCOSE	TOTAL SUGAR	FRUCTOSE	GLUCOSE	TOTAL SUGAR	FRUCTOSE / "GLUCOSE"	FRUCTOSE / TOTAL
				I. Tubers grown on sandy loam							
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*		
Freshly dug tubers	Aug. 30	84.6	15.3	6.7	3.6	10.3	43.6	23.4	67.1	1.86	0.65
	Nov. 3	79.8	20.1	9.9	5.0	15.0	49.5	25.0	74.6	1.97	0.65
	Dec. 20	81.5	18.4	6.3	5.7	12.1	34.5	31.1	65.6	1.11	0.52
	Jan. 30	81.2	18.7	6.5	6.3	12.8	34.7	33.7	68.5	1.03	0.51
	Mar. 22	83.4	16.5	6.0	5.1	11.1	36.6	30.8	67.4	1.19	0.54
Tubers stored at 32°–35° F. Relative humidity 89–92	Dec. 20	78.7	21.2	7.9	5.7	13.6	37.4	26.9	64.3	1.39	0.58
	Jan. 30	77.6	22.3	8.0	7.0	15.1	36.0	31.7	67.8	1.14	0.53
	Mar. 22	76.2	23.7	8.5	6.1	14.7	36.0	26.0	62.0	1.39	0.58
Tubers stored at 33°–44° F. Relative humidity 89–92	Dec. 20	78.0	21.9	8.0	5.9	13.9	36.8	26.9	63.7	1.37	0.58
	Jan. 30	77.9	22.0	8.2	7.3	15.5	37.2	33.1	70.4	1.12	0.53
	Mar. 22	76.7	23.2	8.9	5.7	14.6	38.4	24.4	62.9	1.57	0.61
Tubers stored at 34°–49° F. Relative humidity 74–86	Dec. 20	76.8	23.1	8.5	5.6	14.1	36.9	24.2	61.1	1.52	0.60
	Jan. 30	74.2	25.7	9.3	7.7	17.0	36.4	29.9	66.4	1.22	0.55
	Mar. 22	69.0	30.9	10.5	7.6	18.2	34.1	24.7	58.9	1.38	0.58
				II. Tubers grown on silt loam							
Freshly dug tubers	Aug. 30	83.4	16.5	8.5	3.5	12.0	51.8	21.3	73.2	2.42	0.71
	Nov. 10	78.6	21.3	10.9	5.7	16.6	50.9	26.7	77.6	1.91	0.65
	Dec. 20	80.3	19.6	7.9	5.1	13.0	40.2	26.0	66.3	1.55	0.61
	Jan. 30	82.3	17.7	6.7	5.9	12.7	38.0	33.7	71.8	1.12	0.53
	Mar. 22	82.3	17.6	6.4	5.0	11.4	36.1	28.4	64.6	1.27	0.56

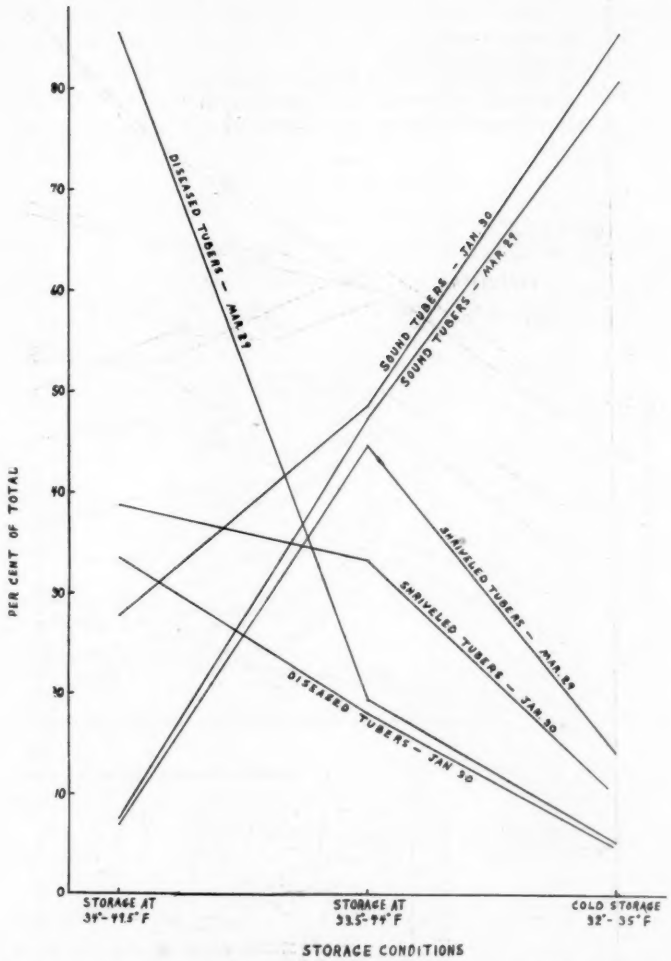

FIG. 1. Per cent. sound, shriveled, and diseased girasole tubers stored under various conditions.

Moisture content.—Prior to the storage period, November 3, there was an increase in dry matter, as shown in figure 2. From then until March 22, the tubers kept in the ground showed a steady increase in moisture. Those kept in a root-cellar at 34°–49° F. and relative humidity of 74–86 per cent. showed a consistent decrease in moisture, totaling 10.8 per cent. Those stored at 33°–44° F. and 89–92 per cent. humidity lost only 3.1 per cent., and those at 32°–35° F. and 89–92 per cent. humidity lost 3.5 per cent.

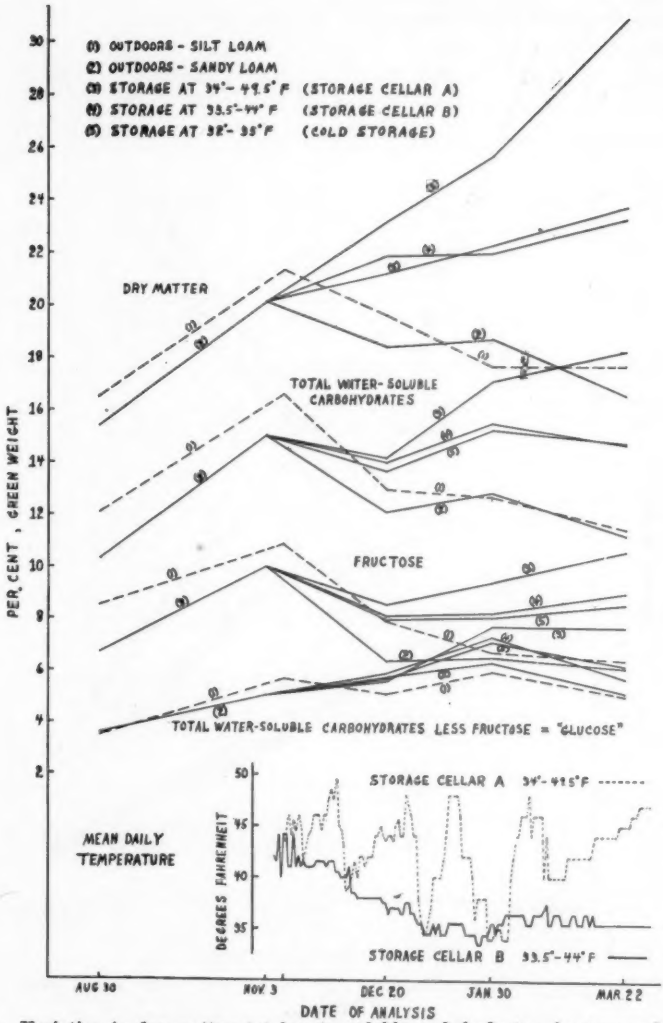

FIG. 2. Variation in dry matter, total water-soluble carbohydrates, fructose and "glucose" in girasole tubers under various storage conditions. University Farm, St. Paul, Minnesota.

Carbohydrates.—The total water-soluble carbohydrates are built up in the tuber until November, when a maximum is reached, both on the green and dry weight basis. This is shown in table III, and figures 2 and 3.

From November to March there is a total decrease in water-soluble carbohydrate on a dry weight basis. Between November 3 and March 22, there is a loss of more than 10 per cent. of the water-soluble carbohydrates on a dry weight basis. There are minor fluctuations which cannot be explained by the data presented. The rise in water-soluble carbohydrates on January

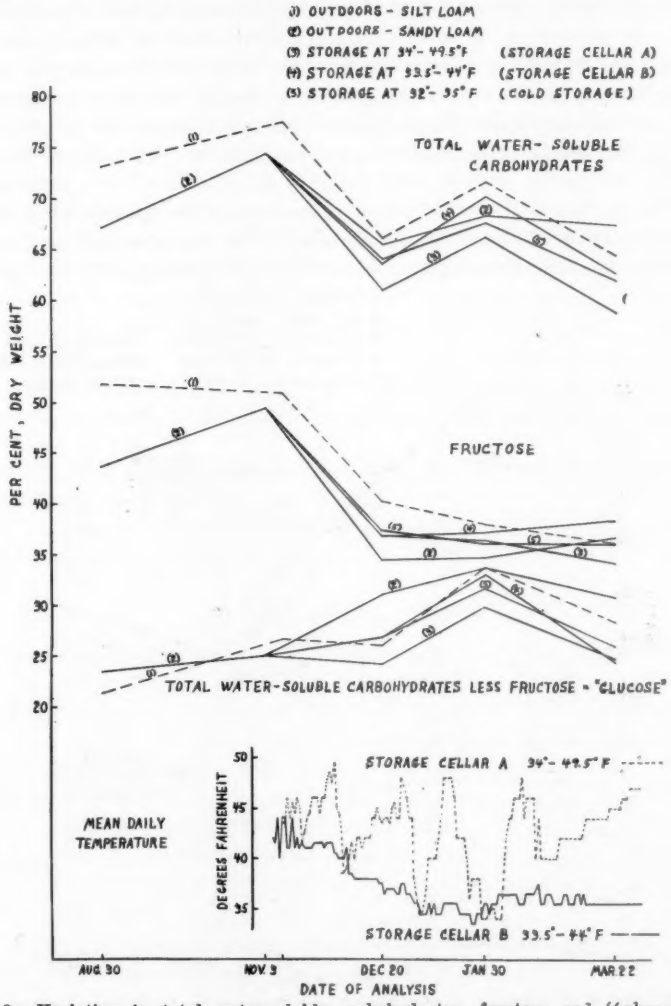

FIG. 3. Variation in total water-soluble carbohydrates, fructose and "glucose" in girasole tubers under various storage conditions.

30 may be due to the seasonal difference in the material. TRAUB (11) has shown that there is a difference in the carbohydrate fractions extractable from apple wood at various seasons of the year. It will be noted that the decline in fructose is consistent, and that the fluctuations in water-soluble carbohydrates is due to other unexplained causes.

In storage the continual loss of moisture apparently has a marked effect upon the percentage of total sugars per unit green weight, as shown in fig. 3. Under the more favorable storage conditions the moisture content and percentage total sugars per unit green weight are more constant.

From the standpoint of possible fructose manufacture the ratios of fructose to other sugars, and fructose to total sugars are of primary importance. During the winter, both in storage and in the field, there is a simultaneous increase in "glucose" and decrease in fructose, although the latter process is the greater as shown in figures 3 and 4. The total sugar content reaches a maximum at the beginning of November, and consequently the best time

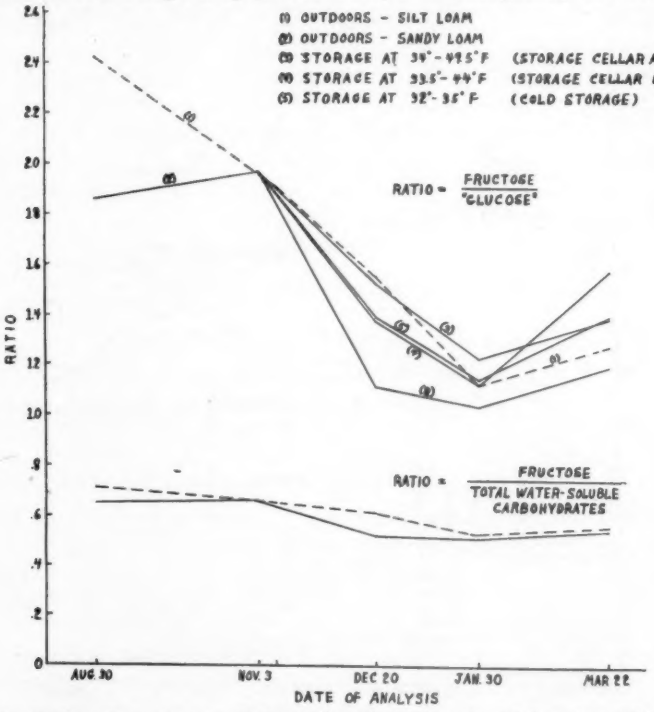

FIG. 4. Ratios of fructose to "glucose" and fructose to total water-soluble carbohydrates, in girasole tubers under various storage conditions.

to utilize the tubers for the maximum fructose yield per unit weight is at this stage when the ratio of fructose to other sugars is the highest.

Conclusions

1. Girasole tubers in storage under the conditions of the experiment have a larger amount of water-soluble carbohydrates per unit green weight than tubers left in the ground over winter.

2. Girasole tubers may be successfully stored as a truck crop, and as propagation stock, at a temperature range of 32°–35° F., and a relative humidity of 89–92 per cent. The optimum conditions for storage, which may or may not approximate the conditions described above, are still to be determined.

3. Tubers stored at temperatures above 40° F. lose moisture rapidly, shrivel, and are subject to storage diseases to such an extent that practically the entire crop so stored is lost by the end of the storage period.

4. From the time of maturity in the fall up to the end of January there is a consistent decrease in the ratio of fructose to glucose, and of fructose to total water-soluble carbohydrates under all conditions studied. From the standpoint of possible fructose manufacture, harvesting and utilization should take place near the time of maturity in November under Minnesota conditions.

5. There is apparently a seasonal variation in the proportion of the water-soluble carbohydrates extractable.

6. Girasole tubers at the time of harvest do not contain appreciable amounts of free reducing sugars.

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SEASONAL VARIATION OF THE CHLOROPLAST PIGMENTS IN SEVERAL PLANTS ON THE MALL AT WASHINGTON, D. C.[1]

F. M. SCHERTZ

Introduction

The following investigation was undertaken to determine if possible what effect the time of year might have upon the chloroplast pigments. It is of course known that the time of year influences the nutrient elements which are found in the soil and these in turn affect the pigments which are present in the leaves. Plants were chosen, to which no chemical fertilizers were added during the course of the year. It was thought that this experiment might possibly aid in interpreting the results obtained from crop plants which had been fertilized.

Material used

All of the leaves (without petioles) used in these experiments were weighed, ground and extracted immediately after picking. A ten gram sample was used in each case while the extraction and the fractionation of the pigments were done according to methods[2] already described by the writer. All determinations were made colorimetrically.

The first collection was made from very young leaves as soon as the required amount could be easily obtained and the last collection was made just before frost killed the leaves. The pigments as given in table I are the result of only one determination for each pigment on a single ten gram sample of leaves. Three trees and two shrubs were chosen for the experiment because of the different habits of their growth. The samples were always collected from the same side of the plant, and whenever possible the leaves were picked from the same branch. The data were obtained during the year 1920.

Results

The results of the investigation are presented in table I where the date of the leaf collections and the species of plant investigated are shown.

In looking over the results, it is very difficult to draw any very definite general conclusions regarding the pigments present in these plants. In drawing conclusions from work of this type, one must always consider the

[1] Soil Fertility Investigations, United States Department of Agriculture, Washington, D. C.

[2] SCHERTZ, F. M. The extraction and separation of chlorophyll ($\alpha + \beta$), carotin and xanthophyll in fresh leaves preliminary to their quantitative determination. Plant Physiol. 3: 211–216. 1928.

TABLE I

THE CHLOROPLAST PIGMENTS (IN MILLIGRAMS PER 10 GRAM SAMPLE OF FRESH LEAVES) PRESENT IN LEAVES FROM SHRUBS AND TREES GROWING ON THE MALL AT WASHINGTON, D. C.

PIGMENTS	*Buxus sempervirens*																	
	YOUNG LEAVES									OLD LEAVES								
	July 1	July 20	August 3	August 17	August 30	September 13	September 27	October 11	October 28*	July 1	July 20	August 3	August 17	August 30	September 13	September 27	October 11	October 28*
Carotin	0.93	0.85	0.88	1.00	0.60	1.00	0.75	0.88	0.87	0.96	0.80	0.63	0.83	0.78	0.85	.....	0.71	.....
Xanthophyll	1.50	2.00	1.40	2.30	3.00	2.80	2.80	2.80	2.70	1.90	2.40	1.40	1.50	3.00	2.40	.....	1.40	.....
Total carotinoids	2.43	2.85	2.28	3.30	3.60	3.80	3.55	3.68	3.57	2.86	3.20	2.03	2.33	3.78	3.25	.....	2.11	.....
Chlorophyll (α+β)	14.1	13.6	18.7	17.0	16.2	18.7	18.0	17.6	21.5	13.6	12.6	15.0	13.1	14.8	13.6	.....	14.8	.....
	Ginkgo biloba									*Prunus cerasifera*								
Carotin	1.10	1.00	1.10	0.96	0.86	0.73	0.73	0.48	0.32	1.50	0.96	1.00	1.20	0.93	1.00	1.03	0.45	0.66
Xanthophyll	1.60	2.40	2.10	2.10	2.40	2.30	1.70	1.90	2.30	2.80	3.70	3.20	2.80	2.00	2.70	2.40	0.50	4.00
Total carotinoids	3.70	3.40	3.30	3.06	3.26	3.03	2.43	2.38	2.62	4.30	4.66	4.20	4.00	2.93	3.70	3.43	0.95	4.66
Chlorophyll (α+β)	16.0	23.0	18.2	16.0	14.8	14.5	15.6	**	**	21.1	27.5	24.3	23.0	18.7	18.7	24.1	**	**
	Acer palmatum									*Chaenomeles legenaria*								
Carotin	1.50	0.80	1.50	1.40	1.40	1.40	1.40	1.30	1.20	1.40	1.00	1.30	1.10	1.00	0.73	0.96	0.56	1.60
Xanthophyll	3.10	3.50	3.20	3.30	1.00	3.40	3.40	2.60	3.00	3.46	3.10	2.10	2.50	2.10	2.20	2.00	2.40	2.60
Total carotinoids	4.60	4.30	4.70	4.70	2.40	4.80	4.80	3.90	4.20	4.86	4.10	3.40	3.60	3.10	2.93	2.90	2.96	4.20
Chlorophyll (α+β)	25.0	27.5	24.0	20.7	23.5	32.0	32.0	27.5	34.0	17.7	18.2	16.6	16.0	17.6	14.1	18.7	18.7	**

* All of the leaves of these plants were noticeably yellow except those of the *Gingko*.
** These chlorophyllin solutions were all of a dirty green color indicating that the chlorophyll had been broken down somewhat.

errors which are ever present in the methods used in making the determinations. The errors which were made in estimating the carotinoids, as has been shown in previous papers which described the colorimetric methods that were used here, are much larger than were the errors made in estimating the total chlorophylls. The chlorophyll data may then be considered of most value.

It is interesting to note that the amount of chlorophyll present in the three trees rises on July 20 and then there apparently is a rapid drop for a month or more and then later a rise in all but the *Ginkgo*. Perhaps the fact that this species belongs to an entirely different group of plants might account for its different behavior. In the case of the boxwood, *Buxus sempervirens,* young leaves (1920 growth) and old leaves (1919 growth) were used in the experiments. The old leaves contained much less chlorophyll than the young leaves. The amount of chlorophyll in both types of leaves apparently increased as the season advanced. The amount of chlorophyll present in the Japan quince evidently increased also as the season advanced. There is no explanation offered as to why the shrubs behave differently than the trees.

It can be said about the carotinoids that two of the trees and the Japan quince contained more pigment at the beginning of the season than did the boxwood and the *Ginkgo*. The maple leaves contained more pigment and there was less variation in the pigment content of this tree during the course of the season than that of any of the other plants investigated. The carotinoids present in *Prunus* decreased to a minimum the first of September and then gradually increased again. The *Ginkgo* leaves showed a gradual decrease in the amount of the carotinoids present in them. On the whole, the old leaves from the boxwood contained less yellow pigments than did the young set of leaves, although the young leaves started the year with less pigment.

The chlorophylls and the carotinoids may be correlated in a few cases. *Acer palmatum* contained the greatest amount of both pigments. The amounts of the green and the yellow pigments present in *Ginkgo* decreased as the season advanced. Both the greens and the yellows decreased and then increased in *Prunus cerasifera*. In a rough way the carotinoids present in the Japan quince parallels the amount of chlorophyll present. In general, it may be said that in every case the amount of carotinoids roughly parallels the amount of chlorophyll present in the leaves.

The investigations of WILLSTÄTTER and STOLL[3] have shown that the chlorophyll content of some of the plants which they worked with varied considerably during the growing season.

[3] Willstätter, R., and Stoll, A., Untersuchungen über Chlorophyll. 1913.

Table II shows the results of their investigation. Nothing is known regarding the conditions of growth of these plants. These conditions undoubtedly were a very important factor with reference to the amount of chlorophyll which was found in the leaves.

TABLE II

THE AMOUNT OF CHLOROPHYLL PRESENT IN PLANTS AT DIFFERENT TIMES DURING THE GROWING SEASON

PLANT	DATE	MILLIGRAMS OF CHLOROPHYLL PER TEN GRAMS OF FRESH LEAVES
Stinging nettles	June 23, 1911	17.4
	July 20, 1911	22.3
	March 22, 1912	12.6
Plane tree	June 1, 1911	15.3
	July 25, 1911	24.9
Horse-chestnut	June 8, 1911	28.3
	July 22, 1911	34.6

Discussion

In a paper of this kind great care must be exercised to avoid drawing conclusions that are not warranted. There are many chances for error and the investigator must always be on his guard.

To secure data which would be of great value, an investigation such as this one should be continued during the course of several seasons and all meteorological data should be correlated with the quantitative estimation of the pigments. The data from a single season will show only what happened during that one year and of course cannot be relied upon for general conclusions.

Moreover, samples should be analyzed in duplicate so as to detect and eliminate any possible errors in the quantitative separation of the pigments. This would greatly increase the amount of work to be done but it is believed that the results obtained would more than justify the labor required. Caution must be exercised in the taking of samples. The method of sampling must be carefully planned in advance so that it will not be necessary to revise the scheme, for leaves picked from different plants or from different parts of the same plant vary greatly in pigment content. The error in sampling may easily be greater than any errors due to the method of extraction and estimation. Investigations conducted so as to show the pigment content of the leaves from various parts of the same plant would be exceedingly interesting.

In the collection of the leaves, care must be used that the leaves contain unaltered chlorophyll; for if the green pigments are altered in any manner the colorimetric results may not be the true values at all for the chlorophyll. The chief danger with which the investigator must contend is the rapid change during the autumn when the leaves are changing color. Some of the results obtained in this investigation had to be discarded because of autumnal changes which affected the green pigments. Other factors may affect the color of the leaves even during the actively growing season.

Soil conditions also should be carefully noted, for these have been shown to have a very decided influence on pigment formation.

The most valuable data would be obtained if each factor could be artificially controlled. The most important factors influencing the quantity of pigments present are rainfall, soil moisture, nutrient elements in the soil, light, temperature and humidity.

Using Weather Bureau reports, an attempt was made to correlate the effects of temperature, sunshine and rain upon the amount of the chloroplast pigments present in the leaves. It seemed doubtful that any correlations could be established between environmental conditions and the amount of pigments produced from the results of one season's work.

The results obtained in this study show that there is considerable fluctuation in the amount of chloroplast pigments present in green leaves even during the normal growing season. The data presented should lead to a closer study of all the factors which affect the amount of the chloroplast pigments in growing plants.

UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

EFFECTS OF STORAGE ON ALCOHOLIC EXTRACTS OF PLANT TISSUES. AMINO ACID CHANGES[1]

JAMES E. WEBSTER

(WITH ONE FIGURE)

Plant chemists, as well as others, are confronted with the necessity of storing much of the material they desire to analyze, since usually a large number of samples are taken at one time. Three major methods are in vogue for this purpose: preserving in alcohol, freezing, and desiccation by heating to various temperatures. Various reasons have been advanced for the use of each, but only in the case of desiccation by heating (**3, 4**) has extended study been made of the influence of the method upon the distribution of the various constituents. APPLEMAN and ARTHUR (**1**) report on the effects of storing in alcohol upon certain of the carbohydrates, but apparently little more work has been carried out upon the effects of this very important method upon the extracted constituents. As a result of this scarcity of information it is proposed to begin a series of studies on the effect of storage in alcohol upon the major plant constituents.

While it has been recognized for some time that to make a comprehensive study of the nitrogen fractions in plants, it was necessary to work with fresh material, still many workers have found it convenient to study certain of these fractions in samples preserved in alcohol. Chief among these is the alpha amino acid fraction, and it is this fraction which will be studied in the present work.

Experimental

Preserving.—All of these samples were prepared by boiling the green plant tissue in 95 per cent. aldehyde-free alcohol, to which had been added an excess of $CaCO_3$. After boiling for half an hour, the material was cooled and filtered. The alcohol was then expressed from the tissue as thoroughly as possible by hand, and the residue discarded.

Before filtering, a small amount of KNO_3 was added to solution (B) and 1 gram of asparagine was added to the 1800 cc. of solution (D).

Samples.—Solutions (A) and (B) were prepared from commercial samples of spinach and were stored in January. They were alike with the exception of the nitrates added to solution (B). Solutions (C) and (D) were also prepared from market spinach, but these were stored in August. They were alike excepting for the asparagine added to solution (D). Solu-

[1] Published with the permission of the Director of the Oklahoma Agricultural Experiment Station.

tion (E) was prepared from young alfalfa, and was started in September.

Storage.—Samples (A), (B) and (E) were stored in two liter flasks, sealed to prevent evaporation between samplings. They were placed in a warm spot in the laboratory and exposed to daylight. Samples (C) and (D) were made up to a given volume and then transferred to individual volumetric flasks, sealed and stored in the dark until each determination was to be run.

Analysis.—One hundred-cc. samples of the extracts were evaporated to a small volume several times on a water-bath to remove alcohol, then transferred to 50-cc. volumetric flasks and 2-cc. aliquots were used for each determination. The micro Van Slyke apparatus was used and the procedure outlined in Mathews Physiological Chemistry followed. The results are expressed in terms of mg. of amino nitrogen in 2 cc. of solution and are shown in fig. 1. No allowance was made for ammonia or amide nitrogen in calculating the percentages.

Discussion

In reviewing the graph it is at once apparent that there is a marked decrease in the percentage of amino nitrogen in every case. It is also apparent that the decrease is not uniform in every case; in fact, in sample (C) where the solution was stored in individual flasks there is considerable fluctuation, indicating that the solution was acting differently in the various flasks. It is also worth noting that solutions (B) and (E), high in nitrates, show the greatest changes. However, nitrates were determined on (B) along with the amino acid determinations and showed no change. This seems to indicate, then, at best, only a casual relationship.

Very probably the reaction takes place in the same manner as that occurring in a water solution as shown by DAKIN and DUDLEY (**2**) in which they were able to demonstrate the formation of ammonia from amino acids as shown in the following equation:

$$\begin{array}{l} CH_3 \\ | \\ CH \cdot NH_2 \\ | \\ COOH \end{array} \longrightarrow \begin{array}{l} CH_3 \\ | \\ C=O + NH_3 \\ | \\ CHO \end{array}$$

If this represents the true reaction taking place, we would expect an increase in the reducing power of the solution and an increase in ammonia. This problem is to be started at an early date and perhaps then we can say more regarding the mechanism of the change. There is also the possibility of some enzyme action bringing about a deaminization, although this is contrary to the common belief that preservation in alcohol stops enzyme action.

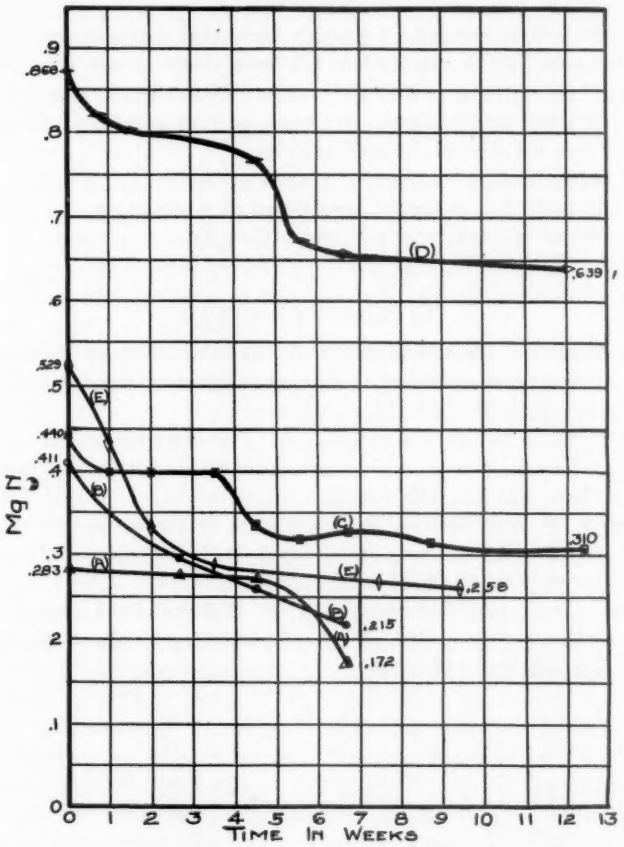

FIG. 1. Graphic representation of the changes in amino acid nitrogen of plant extracts during storage in alcohol.

(A) Spinach extract, stored in January, in warmth and light.
(B) Spinach extract + nitrate (KNO_3), stored as (A).
(C) Spinach extract, stored in August, in darkness.
(D) Spinach extract + asparagine, stored as (C).
(E) Young alfalfa extract, stored in September, in warmth and light.

Neither should one overlook the possibility of certain substances catalysing the changes. Work on the influence of nitrates, phosphates, and possibly other groups, will be reported later.

Summary

We are able to conclude from this work that there are some, possibly many, changes taking place when alcoholic solutions are stored for long periods; if this applies as well when the solutions are allowed to remain in contact with the plant material, we must be very careful in drawing conclusions from the analysis of such samples.

The author desires to express his appreciation to Dr. BURRELL, of Ohio State University, for his timely suggestions on this problem.

DEPARTMENT OF AGRICULTURAL CHEMICAL RESEARCH,
OKLAHOMA AGRICULTURAL AND MECHANICAL COLLEGE.

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X-RAY PHOTOGRAPHY OF MINERAL ACCUMULATIONS IN PLANTS

C. L. CRUTCHFIELD

(WITH SIX FIGURES)

The accumulation of mineral salts in plants has heretofore been demonstrated mainly by microchemical means, a method which is not always satisfactory for quantitative ends, since some of the minerals may be masked through organic combinations, and since the amount of color produced, in reactions dependent on colors, is not necessarily proportional to the amount of mineral present in the tissues.

In recent years HOFFER[1] and his coworkers have used the microchemical method to demonstrate the accumulation of iron and other elements in the nodal tissues of *Zea mays* in connection with corn root rot problems. The writer has employed an entirely different method, and has been able to demonstrate such mineral accumulations in the nodal tissues of sugar cane, *Saccharum officinarum*, with results that offer beautiful confirmation of HOFFER's discoveries with the microchemical methods in corn.

The purpose of this paper is to present a few facts concerning the method of detecting mineral accumulations by means of X-rays. It is hoped thereby to stimulate more work along this line, and to encourage the use of this method in studies involving mineral deposition, and mineral translocation in plants. As time goes on X-rays have found an ever widening field of usefulness in science, industry, and medicine. It seems quite probable that many other uses for these rays in biological work may be found.

GEORGE L. CLARK, in his "Applied X-Rays," mentions several of the less well-known applications, and says: "Although X-rays because of their short wave-length are much more able to penetrate matter than ordinary light, still they are differently absorbed by different substances; that is to say, all materials are not equally transparent to X-rays." This fact is the basis of the science of radiography. Broadly defined, the technique consists in passing a beam of X-rays through the object to be examined, and by means of a fluoroscope, or preferably a photographic plate, record the varying intensities of the emergent rays, thereby obtaining a shadow picture of the interior of the object. The first practical uses of the X-rays were probably of a radiographic nature, and today radiography is a most useful tool for the medical and industrial diagnostician.

[1] HOFFER, G. N., and CARR, R. H. Accumulation of aluminum and iron compounds in corn plants and its probable relation to rootrots. Jour. Agr. Res. 23: 801–824. 1923. See also, HOFFER, G. N. Testing corn stalks chemically to aid in determining their plant food needs. Purdue Agr. Exp. Sta. Bull. 298. 1926.

The writer has simply employed the essential quality of the X-rays of being differentially absorbed by different substances, in detecting the localization of mineral depositions in plants. In developing the technique, some substances other than those found abundantly in plants were used, mainly to afford substances differing in opacity to the X-rays. Some of the chemicals used are quite opaque to X-rays, others relatively easily penetrated.

In performing the experiments, solutions of 1–2 per cent. strength were used, of the following salts: Ferrous sulphate, aluminum chloride, potassium sulphate, sodium chloride, lead nitrate, and barium chloride. The heavier metals, lead and barium, are quite opaque; following these are iron and aluminum, relatively considerably less opaque; finally, sodium and potassium are very easily penetrated by X-rays.

Normal average sugar-cane sticks were cut at the surface of the soil, the cut end placed at once into a beaker containing one of the mentioned solutions; control sticks were similarly placed in tap water. All sticks were allowed to remain in the solutions for about 48 hours before they were photographed. During this time, continuing transpiration and existing saturation deficits caused the absorption of some of the solution, which could easily be detected from the decrease of volume of solution remaining in the beakers.

The sticks were now laid across the film-holder containing an X-ray film, and were exposed to X-rays emanating from a Coolidge X-ray tube. In order to secure good photographs, the rays must be adjusted to a suitable penetrating power, and the time of exposure must also be adjusted to the results desired. The photographs reproduced in this paper, were made with a voltage of only about 27500, with 10 milliamperes of current. The exact voltage used depended somewhat upon the thickness of the sticks of cane. The time of exposure was usually about 3.5 minutes. The cane was placed as close to the film-holder as possible in order to obtain clear sharp images. In any given case the investigator will find it necessary to carry on preliminary experiments with different milliamperages and voltages with each object of research, to obtain the best results. In general, the voltage will be much lower than is easily obtained in most makes of apparatus, the average medicinal unit being designed for higher voltages.

The distance from the X-ray tube to the film is, of course, very important since the length of exposure varies with the distance. The photographs shown here are the results of a preliminary study. In fig. 1 is shown a stick which was allowed to take up barium chloride through the cut end. The barium was deposited most heavily at the node, or under the buds. Not only are the nodal deposits shown, but also the vascular system is clearly outlined. It shows better in the photograph than in the reproductions from it. One can see some of the root bands also, rather easily.

FIG. 1. Sugar cane stick, photographed by X-rays after having taken up barium chloride. Dark bands show nodal accumulation of $BaCl_2$.

Fig. 2 was made from the photograph of a stalk allowed to absorb lead nitrate solution. The control stick is at the left. The root bands show very

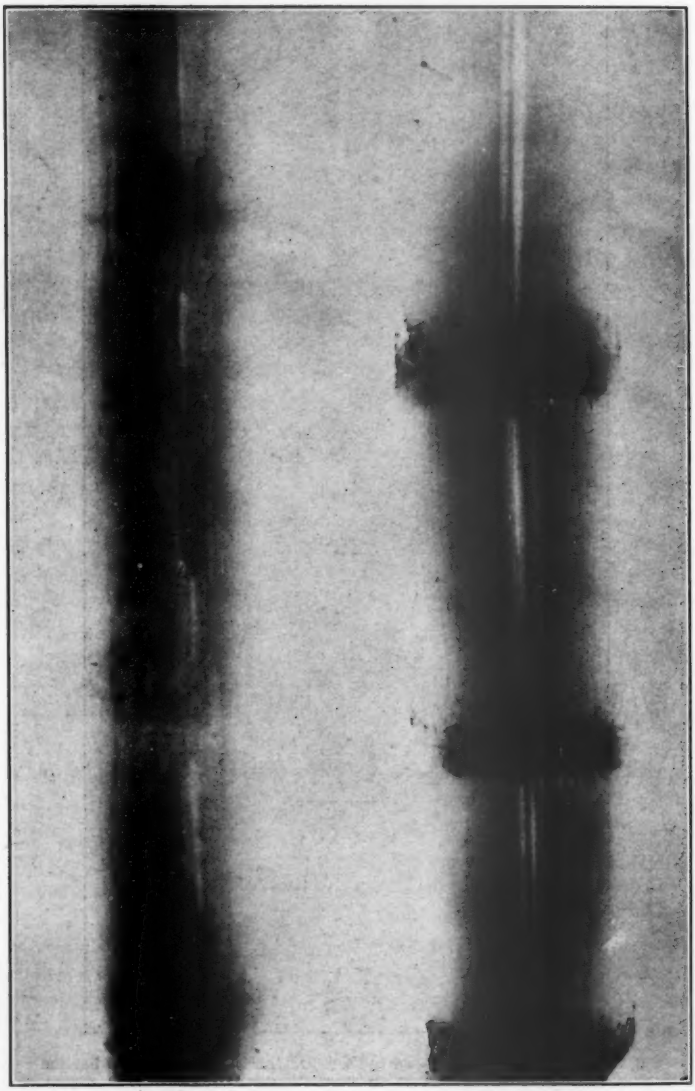


FIG. 2. X-ray photograph of sugar cane sticks; at the right, treated with lead nitrate; at the left, the control. The lead nitrate is very prominent in the root bands.

FIG. 3. Nodal accumulation of iron, from $FeSO_4$. Improperly exposed.

clearly here, and the vascular system in the internodal region is brought out quite well. In the control sticks two buds are seen at the nodes. Both figures 1 and 2 are made from stalks containing the most opaque salts used. Lead and barium are not very prominent constituents among the ash elements of plants, though probably never completely absent. Barium is rather universally distributed in soils and plants, but these elements do not occur in such abundance as to show up in the nodes of the control sticks. Iron and aluminum, however, are nearly always fairly abundant in soils. In fig. 3 is shown a photograph from an improperly conducted exposure of a stalk which had been treated with $FeSO_4$. The nodal accumulation, in spite of the improper exposure, is clearly shown, and even the vascular bundles can be seen. Better examples of iron accumulation are shown in fig. 6.

The lighter elements, potassium and sodium, are more readily penetrated by X-rays, and so appear to clear up the nodes. In fig. 4 we see a stick which was allowed to take up NaCl. The control stick, to the left, contrasts strongly with the treated one. The latter seems to have deposited the sodium salt sharply at the node. One might question whether it is the sodium, or chlorine, or both that are involved in the deposition. In the treated stick (right) one can see some spots of lighter color. These were found, on cutting the sticks, to be the cavities produced by a stalk borer.

In fig. 5 the control stick is to the right, and the treated stick (left) was allowed to absorb K_2SO_4. This also seems to clear out the nodal tissue, and to make it less dense than normal. The proper interpretation of this clearing of nodes by Na and K salts probably is that the sodium or potassium displaces some heavier metals, as Fe and Al, that normally are deposited in that region, and so prevents their union with the organic constituents of the nodes. These X-ray results are again in harmony with HOFFER's results in which he claims that potassium prevents the over-accumulation of iron or aluminum in the nodes of corn.

The final figure 6 is most interesting. These stalks were not allowed to absorb solutions, but were taken from soils in which the soluble mineral content was high. The photographs show what can be accomplished in checking up on soil experiments. The small stick to the right is a young cane grown on a soil containing high available aluminum, while the two to the left of it are from a soil with high available iron. The stalks from the iron containing soil showed slight indications of iron injury, but yet growth was practically normal. Tests made for iron and aluminum by other methods did not show them present, but the X-ray photographs clearly show the nodal accumulations. This method may therefore exceed in sensitivity the microchemical methods of detecting these accumulations. A very interesting feature of the stalks grown on the iron-rich soil is the clear indication in

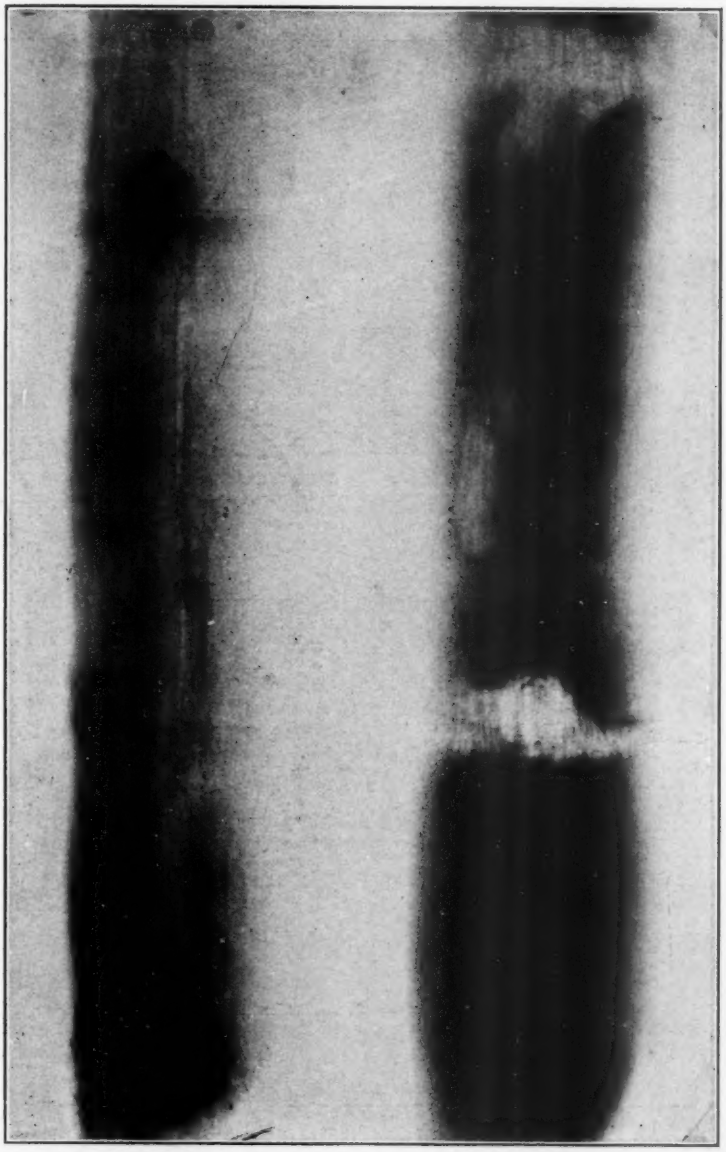


FIG. 4. Sugar cane sticks: Right, after exposure to NaCl, nodal accumulation being indicated by clearer nodal tissue; left, control. Spots in the experimental stick are due to a borer.

FIG. 5. Sugar cane sticks: Left, treated with $FeSO_4$; right, control. Nodal accumulation is indicated by clearer nodal tissue.

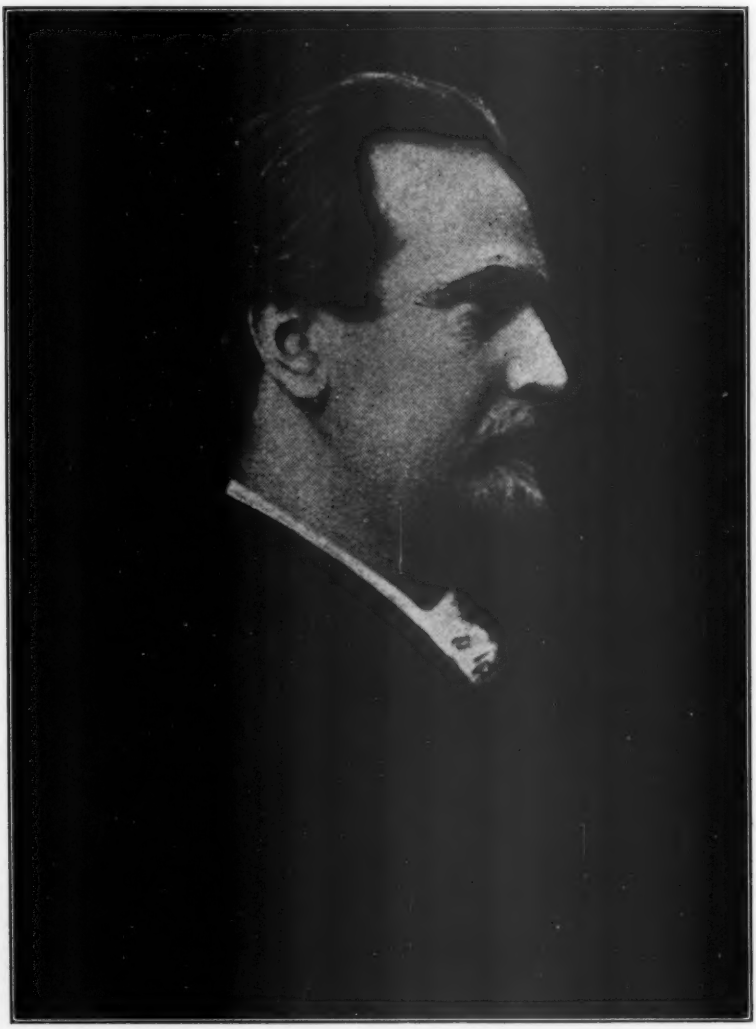
FIG. 6. Sugar cane sticks grown on soils with high available minerals. Right, grown on high available aluminum soil. Center and left, on high available iron soils. The nodal accumulations are easily seen, as well as the vascular tissue of internodes. See text for additional details.

the middle stalk of fig. 6 of three bands of iron deposition at the nodes, one across the central part of the node, and one on either side. Professor HOFFER, who has examined these photographs, has found similar banded nodal deposits by microchemical means.

Additional work is in progress with the X-ray method in the hope that some of the problems connected with the translocation of salts in plants may be solved. It ought to be possible to determine whether xylem or phloem is mainly concerned in translocation of salts by a study of localization of minerals in plants which have absorbed the salts through unbroken root systems. Space does not permit the inclusion of photographs of some other plants, such as corn, tomato, and some quite good ones of *Selaginella* which seem to show the structure of the vascular system very well.

In conclusion, the writer desires to express his thanks and appreciation to Mr. W. S. KENDRICK, of the Victor X-Ray Corporation, Chicago, for the loan of apparatus, and the use at times of their X-ray equipment and dark room, in connection with the more recent work. He is also indebted to Professor CHARLES A. SHULL for assistance in preparation of the manuscript.

HONOLULU, HAWAIIAN ISLANDS.



JULIUS VON SACHS

BRIEF PAPERS

JULIUS VON SACHS

(WITH ONE PLATE AND ONE FIGURE)

JULIUS VON SACHS has been called the Father of Plant Physiology both on account of his importance in the development of the science and because he was the first teacher to hold a position which recognized plant physiology as a separate subject in university teaching. An estimation of the important position of SACHS in the development of plant physiology was given in a review of his life by F. NOLL in the Naturwissenschaftliche Rundschau **12**: 460–464, 472–475. 1897. This was in part translated by J. M. COULTER in the Botanical Gazette **25**: 1–12. 1897. GOEBEL also gives a review of his life in a supplementary volume of Flora for 1897. This was translated in Science Progress **7**: 150–173. 1898.

The life time of SACHS covers a period of rapid growth of plant physiology. In his laboratories there developed many students who spread the zeal of their teacher and who led to the development of their new science. Among these may be mentioned PFEFFER, KLEBS, DEVRIES, BARANETZKY, BREFELD, MILLARDET, MOLL, NOLL, PRANTL, STAHL, ELFVING, FRANCIS DARWIN, GODLEWSKI, VINES, MARSHALL WARD, GOEBEL, G. KRAUS, MÜLLER-THURGAU, and many other prominent names. SACHS was considered the grand old man of plant physiology by the many German and foreign students of PFEFFER'S laboratories. An introduction to him was a high honor. The stimulation of research in plant physiology in the United States owes much to the introduction of his textbooks.

JULIUS was the third son of GRAVEUR SACHS, born at Breslau on October 2, 1832. His father was an engraver by trade, and from him young SACHS learned perfectly the art of delineation, accuracy of line and color, which was of the greatest value to him in gaining a livelihood during the lean years of his student life and also later when he became a teacher. As a child, SACHS had for companions the sons of the physiologist, PURKINJE, and no doubt he was much influenced by this association. Early in boyhood he made collections of plants and was expert in their classification. On many field excursions with his father he was encouraged in botanical collections in which he took great pride. He gave much of his time between the ages of thirteen and sixteen years to drawing and painting the flowers, fungi, and other specimens which he collected. When he was sixteen years old, his father died, and the next year both his mother and a brother died of the cholera. From this time on, SACHS had to earn his

own education by means of the trade which he had learned from his father.

In the Gymnasium from 1845 to 1850 SACHS enjoyed especially the natural sciences. He made a collection of skulls, and wrote a monograph on the crayfish. His natural science teacher, KROBER, solemnly warned him against devoting himself to the natural sciences.

FIG. 1. JULIUS VON SACHS.

Upon the death of both parents, SACHS was taken into the family of PURKINJE who had accepted a professorship at the university at Prag. He completed the courses of the Gymnasium and was admitted to the university in 1851. In Prag he published a Bohemian scientific journal, Ziva, and to this journal he made a number of scientific contributions.

Upon the completion of his university course in 1856, SACHS began to devote his time to plant physiology, working especially upon seed germination, the digestion and transport of nutrients, and the development of plant

organs. In April, 1859, upon the recommendation of HOFMEISTER, SACHS became assistant to STÖCKHARDT at Tharand. He was quickly advanced to take charge of the laboratory of experimental plant physiology. In 1861 he was elected head of the experiment station for plant physiology at Chemnitz, but went instead to a professorship in the Poppelsdorf Agricultural Academy of the University of Bonn. The six years at this academy were most fruitful for research, and here also was published his Handbook of Experimental Plant Physiology. In 1867 SACHS was called to Freiburg to the chair vacated by the resignation of DEBARY, but one year later he succeeded SCHENK at the University of Wurzburg. Here he had at his disposal a large building for his botanical institute. His laboratory became the center of plant physiological research although the number admitted at one time was limited to ten students. SACHS published in 1868 his Lehrbuch, in 1875 a History of Botany, and in 1882 his Lectures upon Plant Physiology, all books outstanding even to the present day. He died at Wurzburg on May 29, 1897.—R. B. HARVEY, *University of Minnesota.*

AN INVADING POTATO SPROUT

When potatoes are grown in fields infested with Couch Grass (*Agropyron repens*), it is not uncommon to find some of the tubers transfixed by the hard, sharply pointed rhizomes of this persistent grass. A growth phenomenon similar to this in appearance, yet very different in nature, was recently observed by the writer; and as this phenomenon is apparently a very unusual one, it deserves to be included in our botanical records.

A sack of potatoes had been stored in a cellar during the winter months, and toward the close of this period as the days grew warmer and the general level of temperature began to rise, the buds of the tubers became active, and vigorous sprouts were formed. On one of these tubers a sprout was observed that had behaved in a most unusual manner. In its growth it encountered a neighboring tuber, in the side of which a small cavity about 5 or 6 mm. in depth had been made by a wire worm. The growing point of the sprout entered this cavity and then continued its growth into the storage tissue of the potato, until it finally emerged on the opposite side. When it was first discovered the sprout had attained a length of 6.5 centimeters, and a diameter of 8 mm.

The accompanying photograph, figure 1, shows the position of the sprout imbedded in the tuber just as it was found, except that a portion of the tissue has been removed so that the full length of the sprout may be seen. At no point was there any evidence of a coalescence of the cells of the sprout with those of the tuber through which it grew, but the turgid sprout fitted very closely into the tunnel it had made. The surface of the tuber tissue

surrounding the sprout was very smooth, and at the point of emergence there was not the slightest indication of a rupture of the tissues, as would probably have been the case had the expanding cells of the sprout broken through by the mechanical force of growth.

The general appearance of the tissue of the tuber suggested that the cells had been digested by the elongating sprout which was able in this way to excavate the tunnel through which it grew. This further suggests the possibility of a sprout arising on one potato being able to procure a part or all of its nutrition from an adjacent tuber—at least under such circumstances as those here recorded.—P. D. STRAUSBAUGH, *West Virginia University.*

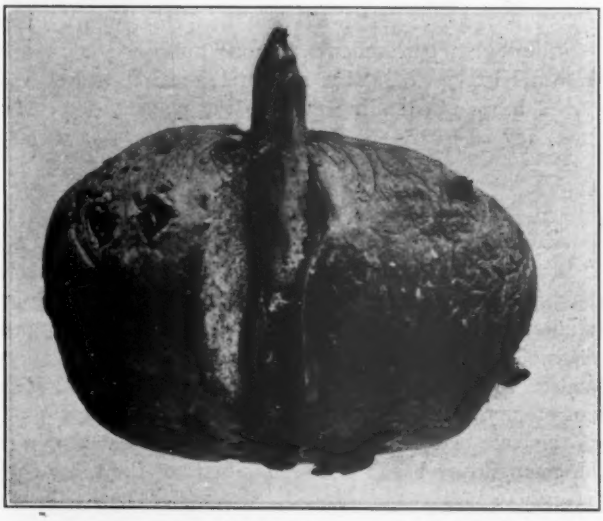


FIG. 1. Potato tuber, penetrated by sprout from a neighboring tuber. The sprout apparently digested its way through the invaded tuber.

SCHLOESING'S EXPERIMENTS ON THE RELATION OF TRANSPIRATION TO THE TRANSLOCATION OF MINERALS

In the last fifteen years a number of papers have been published showing at least partial independence of transpiration and mineral absorption. HASSELBRING (**2**) found that tobacco grown under shade had as high ash content as plants grown in the open. MUENSCHER (**3**) found that doubling the transpiration of barley by decreasing the humidity had no effect on ash absorption. Doubling the transpiration by differences in light intensity nearly trebled the absorption of ash but the ratio of ash to dry matter was

not affected. CURTIS (**1**) has attempted to show that solutes are translocated primarily in the phloem where their movement would of course be unaffected by transpiration.

In spite of this accumulation of evidence, however, SCHLOESING's experiment (**4**) published in 1869 has been so generally accepted in the older texts that its influence persists very generally among teachers of plant science. This note points out certain details in the experimental procedure used by SCHLOESING which have been overlooked in translation, and which would seem to invalidate the conclusions commonly drawn from the data, thus removing the conflict between this and more recent experimental work.

SCHLOESING grew four tobacco plants in large pots, one under a bell jar shaded with muslin for four weeks, and three in the open air for six weeks. The plant under the bell jar transpired 7.9 liters, or 1976 ml. a week while the average transpiration of the exposed plants was 23.3 liters, or 3883 ml. a week.

The dried leaves of the protected plant contained 13.0 per cent. of ash and of the exposed plants 21.8 per cent. at the end of the experiment. After correcting for dry weight and ash content at the beginning of the experiment the total gain of ash was 4.50 and 6.41 grams respectively. The data are claimed to prove the dependence of mineral movement on transpiration.

An examination of the conditions of the experiment shows that the protected plant was not only shaded but received additional CO_2 in undetermined quantities. The results of the experiment can without difficulty be attributed to this difference in the CO_2 supply of the two lots of plants. For example, the rate of gain in dry weight was 10.0 grams a week for the plant receiving additional CO_2 and only 4.9 grams a week for the checks. There was also a rapid accumulation of starch in the plant under the bell jar to a total of 19.0 per cent., while the leaves of the exposed plants contained only 1.0 per cent. of hydrolyzable polysaccharides. The lower per cent. of ash is in large part due to the very rapid accumulation of carbohydrates in the protected plant.

If we consider the rate of ash assimilation we find that the covered plant gained 0.57 grams of ash for each liter of water transpired while the exposed plants gained only 0.28 grams for each liter of transpiration. Also the gain per week in the covered plants was higher in total ash and in all determined elements except chlorine, silica, and iron, and was very much higher in phosphorus and nitrogen.

Summary

The more or less classic experiment of SCHLOESING has been given an unjustified importance in establishing the dependence of mineral movement in the plant upon transpiration. The data are better interpreted

as showing the effects of increased CO_2 rather than of decreased transpiration.—W. E. Loomis, *Iowa State College.*

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NOTES

Fifth Annual Meeting.—The fifth annual meeting of the American Society of Plant Physiologists, held at New York on December 28, 29, and 31, 1929, marks an important milestone in the development of the society. Although the attendance was reduced by the widespread influenza epidemic, and the meeting handicapped by the unexpected absence of both president and vice-president, temporary officers were chosen, and the sessions held as scheduled. The programs were interesting and valuable, as usual, and every one must have felt repaid for being present. The most encouraging feature of the meeting was the detailed report of the secretary-treasurer, Dr. H. R. Kraybill, who analyzed the gains in membership and financial support during the last year. The society continues to gain in numbers at a steady rate, and financial support has increased more rapidly than at any time since organization. The report of the condition of the finances makes it evident that Plant Physiology has not incurred a deficit since its first year; moreover, the unsold volumes now have a value considerably larger than the subsidy which was provided for the first year of publication. It is remarkable that a journal of the high standards of Plant Physiology could become self supporting within the first year of its existence, but the secretary's report shows that this is the fact. Good fellowship marked all of the meetings, and was enjoyed by all. The facilities provided by Columbia University for the meetings were much appreciated.

Life Membership Award.—The committee appointed to choose the third Charles Reid Barnes life member, of which Dr. W. M. Atwood was chairman, selected Dr. Herman Augustus Spoehr, of the Coastal Laboratory of the Carnegie Institution, Carmel, California, for this honor. The selection is a very happy one. Dr. Spoehr has made very valuable contributions to our knowledge of plant physiology. Unlike many biochemists, he knows the living plants thoroughly. His work on carbohydrate metabolism, on respiration, and on photosynthesis, is outstanding work. Dr. Spoehr has been connected with the Carnegie Institution since 1910, and became assistant director of the Coastal Laboratory in 1926. His excellent summary of the process of photosynthesis published in 1926, is the best account in the English language. All members of the Society will feel that the Society has been honored by this award to Dr. Spoehr. He becomes the third link in the chain of life members who form a living memorial in honor of Dr. Charles Reid Barnes, first professor of plant physiology at the University of Chicago.

Stephen Hales Award.—It was expected that the first STEPHEN HALES Prize would be awarded at the New York meeting. However, the committee having this matter in charge, found that it would be advisable to postpone its decision for a time, and announce the first award at the Des Moines meeting in 1929. While this decision may have been a disappointment to some, the delay has some valuable aspects. Certain matters of policy have not been clearly crystallized, and the development of a satisfactory certificate has presented some complications. The additional time will make it possible to meet every problem connected with the award in a satisfactory manner.

Forest Tree Seeds.—Notes have appeared in past numbers of PLANT PHYSIOLOGY regarding sources of seeds for investigational purposes. In this connection, Conyers B. Fleu, Jr., of Germantown, Philadelphia, Pa., has been mentioned, and Otto Katzenstein and Co., of Atlanta, Ga. There are also other sources of seeds of guaranteed origin and quality. The Associated Foresters, Ltd., of Calgary, Canada, advertise seeds of known origin, and will supply along with the seeds, data as to site, stand, and climatic conditions under which they grew. The Brown Co., Berlin, New Hampshire, also advertise forest tree seed of certified origin. It is believed that any of these companies will exercise painstaking care in furnishing seeds for research on the physiology of germination of forest tree seeds.

The U. S. Forest Service has a mimeographed list of forest tree seed dealers, together with a list of the most important U. S. tree species, giving the average number of seeds to the pound, and the dealers who are in position to supply seed of each species.

The Rev. J. Farnsworth Anderson, Glenn Hall, Leicester, England, advertises fresh seed of perennials from all over the world. It is hoped that those needing seeds will find these addresses valuable.

Research Laboratory at Minnesota.—A low temperature research laboratory has been completed at the Minnesota Agricultural Experiment Station for the Section of Plant Physiology. The new installation consists of four refrigerated rooms 9 x 10 x 11 feet, a control laboratory, engine room with 15 horse power compressor, and two controlled greenhouses, with potting sheds. Additional greenhouse space is available also, for cooperation with the Division of Agronomy and Plant Breeding on the winter resistance of field crops, and with the Section of Plant Pathology on pathological physiology.

The refrigeration is by automatic machines of the Lipman type. The rooms are insulated with 12–14 inches of sheet cork. Temperature fluctuations in these chambers is $\pm$ 2° C. with the Absolute Contactor Corpora-

tion regulator, $\pm 0.5°$ C. when the Harvey type thermoregulator is used. Temperatures and humidities are recorded by resistance thermometers and a recorder of the Leeds & Northrup Co. A recording potentiometer for use with photoelectric cells is available for light records.

Fermentation.—SCHOEN, of the Institut Pasteur, has published a monograph on alcoholic fermentation which has just been issued in English translation. It is an unusually lucid and readable account, and summarizes admirably the present status of our knowledge. Especially commendable is the chapter on the role of H^+ in oxidation processes, which gives the clearest discussion of the nature of oxidation in living cells, which the reviewer has seen anywhere. It is published by Chapman and Hall, of London, translated by HIND, and entitled The Problem of Fermentation. The price is 21 shillings.

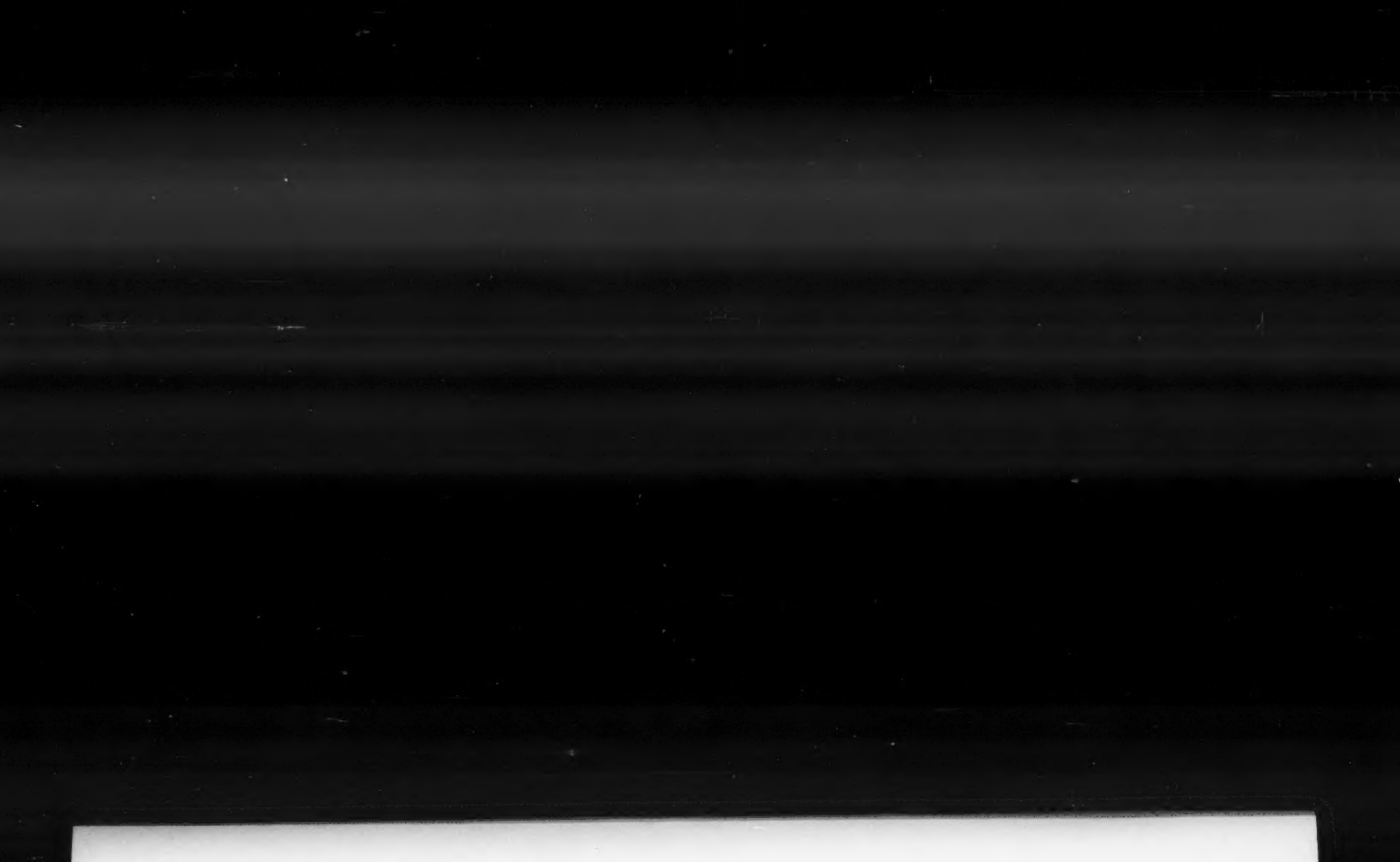
Colloid Chemistry.—The second volume of Colloid Chemistry, theoretical and applied, collected and edited by JEROME ALEXANDER, has appeared from the press of the Chemical Catalog Co., New York. This volume, devoted to Biology and Medicine, contains 57 papers by men of recognized authority in their respective fields, and is a splendid contribution to a fuller understanding of the significance of the colloidal state in living organisms. A few titles will suffice to indicate the unusual nature of this book. There is a discussion of Colloids and X-rays, by Sir WILLIAM BRAGG; Proteins as Colloids, by WOLFGANG PAULI; Inorganic Ferments, by G. BREDIG; The Adsorption of Enzymes, by RICHARD WILLSTÄTTER; Bacteriophage, a Living Colloidal Micelle, by F. D'HERELLE; Colloidal State and Physiological Function, by RUDOLPH HÖBER; The Arrangement and Action of the Colloids of the Plant Cell, by D. T. MACDOUGAL; The Physical Basis of Life, by EDMUND B. WILSON. An appendix contains the Pasteur Lecture delivered in Chicago by JACQUES LOEB on November 24, 1922, in lieu of a paper which LOEB was to have contributed to the volume.

The book is one that every physiologist must read if he is to keep abreast of modern thought on the processes of living. It is a large volume, over 1,000 pages, and costs $15.50; but one could easily spend more and get less in value, as books are now selling. All libraries of biological institutions will find it a necessity.

Numerical Data of Biology.—Attention is called to the publication of several chapters from vol. VI, 1923–1924, of the Tables Annuelles de Constantes et Données Numérique under the title Données Numérique de Biologie et de Physiologie et Chimie Végétales, by E. F. TERROINE and H. COLIN. The tables include bulk chemical compositions of plants; chem-

ical composition of special organs and tissues; content of inorganic, organic and organometallic substances in plants; physical-chemical constants of plant juices; data on gaseous exchanges; and enzyme reactions. It comes in quarto size, 88 pages, from Gauthier-Villars et Cie., 55 Quai des Grands-Augustins, Paris (VI). Bound, the price is 60 francs, 44 francs in brochure form.

Colloid Chemistry of Starch.—The second volume of the Handbuch der Kolloidwissenschaft, edited by W. OSTWALD, is a monograph on starch, Kolloidchemie der Stärke, by M. SAMEC. It contains 16 chapters, dealing with the general characteristics of starch, molecular structure, construction of starch grains, physical characteristics of starch grains, adsorbent properties, paste formation, solution, properties of solutions of starch, protective colloidal action, adsorption of starch by other substances, changes in starch solutions, precipitation of starch, alkali starch, formaldehyde starch, starch esters, and dextrine. The book brings together in excellent fashion the whole field of starch behavior, laying more emphasis upon the experimental results than upon changing theories of starch constitution. It is published by Theodor Steinkopff, Dresden and Leipzig. In brochure form it costs RM 30, and RM 32, cloth bound.

The Alkaloids.—The first part of the second edition of WINTERSTEIN-TRIER'S Die Alkaloide, was published by Gebrüder Borntraeger, Berlin, in 1927. This first part contains 356 pages, and presents the alkaloids whose constitution is fairly well known. The aliphatic nitrogen bases, aromatics, acid amides, urea derivatives, and the heterocyclic bases of the pyrrol-pyridine group occupy this volume. It is replete with interest to those investigating the nitrogenous metabolism of plants. In general the natural plant bases receive too little attention from physiologists. The price of this volume is RM 18.

Colloid Chemistry of Protoplasm.—The first volume of a new series of monographs, Protoplasma-Monographien, is a book by L. V. HEILBRUNN on the Colloid Chemistry of Protoplasm. It is written in English, although published by Gebrüder Borntraeger, Berlin. Several introductory chapters deal with the morphology and chemistry of protoplasm, and methods of study. Then the problems of viscosity, elasticity, temperature and other effects, salt action and electric charges are considered. Other chapters discuss the action of acids and bases, fat solvents, surface precipitation reactions, a specific colloid chemical reaction peculiar to living organisms, and cell division and protoplasmic activity. The book contains 356 pages, and is a fine contribution to the literature of protoplasm. In paper cover, RM 19, bound in cloth, RM 21.